Efficacy of Chemigation Treatments for Reduction of Bacterial Foodborne Pathogens in Preharvest Agricultural Water in Georgia

by

AADEYA ARORA

(Under the Direction of Faith Critzer)

ABSTRACT

Preharvest irrigation water can be a contamination route for fresh produce. This study evaluated peroxyacetic acid (PAA) and chlorine treatment efficacy to inactivate *Salmonella* and STEC in preharvest agriculture water. Surface water collected from two sources in Georgia was inoculated with a cocktail of STEC or *Salmonella*. The water was equilibrated at 12℃ and 32℃ and treated with chlorine (4 and 10 ppm) or PAA (6 and 10 ppm) for 5 or 10 minutes. Bacterial inactivation was analyzed using Analysis of Variance with Tuckey's HSD for significant factors ($p<0.05$). Minimum 5 log bacterial inactivation was achieved for all the treatment combinations. No significant difference was observed at 32℃ for *Salmonella*, whereas 10ppm chlorine or PAA resulted in maximum inactivation of STEC. At 12ºC, 10ppm chlorine or PAA caused the highest bacterial inactivation for STEC and *Salmonella*. PAA or chlorine at 10ppm for STEC were the most efficient treatments.

INDEX WORDS: Preharvest Water, Irrigation, *Salmonella*, STEC, PAA, Chlorine, Produce Safety

EFFICACY OF CHEMIGATION TREATMENTS FOR REDUCTION OF BACTERIAL FOODBORNE PATHOGENS IN PREHARVEST AGRICULTURAL WATER IN GEORGIA

by

AADEYA ARORA

B. Tech., S.R.M. Institute of Science and Technology, India, 2020

A Thesis Submitted to the Graduate Faculty of The University of Georgia in Partial Fulfillment of the Requirements for the Degree

MASTER OF SCIENCE

ATHENS, GEORGIA

2023

EFFICACY OF CHEMIGATION TREATMENTS FOR REDUCTION OF BACTERIAL FOODBORNE PATHOGENS IN PREHARVEST AGRICULTURAL WATER IN GEORGIA

by

AADEYA ARORA

Major Professor: Faith Critzer
Committee: Laurel Dunn
Abhinav Mishra

Electronic Version Approved:

Ron Walcott
Vice Provost for Graduate Education and Dean of the Graduate School
The University of Georgia
August 2023

DEDICATION

This thesis is dedicated to Maa, Papa, and my brother Daksh for their unconditional love and support and for being my pillar of strength. This work is also dedicated to my grandparents for always showering their blessings on me from heaven.

ACKNOWLEDGEMENTS

I would like to start by thanking my parents and my brother for their immense love, unwavering support, and constant encouragement throughout this journey. Your unwavering support has been the bedrock of my academic achievements. Your belief in me, even during moments of self-doubt, has been the driving force behind my perseverance.

I would like to extend my gratitude to my advisor Dr. Faith Critzer for believing in me and providing me with such a great opportunity. It was a wonderful experience learning under your guidance, mentorship, and expertise.

I would also like to thank Dr. Martha Isabel Sanchez Tamayo for helping me through my research project and supporting and guiding me throughout. Many thanks to everyone who is a part of Critzer Lab. I am grateful for your support and presence throughout this journey.

Lastly, I would like to thank my friends back in India for making me believe in myself and being my strength.

TABLE OF CONTENTS

Page

LIST OF TABLES

Page

LIST OF FIGURES

Page

CHAPTER 1

INTRODUCTION

For maintaining a healthy lifestyle, the consumption of fresh produce has been increasing for many years, increasing its production globally (León et al., 2008). Fresh produce is usually eaten raw and involves a minimum amount of cooking which increases the risk of spreading the pathogenic bacteria causing foodborne diseases (Berger et al., 2010). Pathogenic bacteria such as *Escherichia coli* O157:H7, Shiga toxin-producing *E. coli, Salmonella enterica,* and *Listeria monocytogenes* caused major microbial fresh produce outbreaks (Kozak et al., 2013).

There are several steps associated with the produce production pathway, which can serve as an entry point for pathogenic contamination. By following Good Agricultural Practices (GAP), the risk of contamination can be reduced to a certain extent but cannot be eliminated completely (Declan Iwu & Okoh, 2019). Points of bacterial entry can be before or after the harvesting (Alum et al., 2016). Contaminated preharvest irrigation water is one of the initial transmission sources in the produce production pathway from which the pathogenic bacteria can enter the food chain (Uyttendaele et al., 2015a). Irrigation water is subject to contamination as it can contain soil, fecal matter, pollutants, agricultural run-off, and wastewater discharge. Surface water is the most common source of irrigation water, which can contain the above-mentioned contaminants in high concentrations as it is an open source and is constantly in contact with the environment (Uyttendaele et al., 2015a).

Enterotoxigenic *E. coli* and *S. enterica* have been the cause of most foodborne outbreaks. *E. coli* O157:H7 can withstand unfavorable conditions and hence has led to the majority of outbreaks due to irrigation water (Dirk Van Elsas et al., 2011a). In Georgia, 79.2% of surface water samples collected showed the presence of *Salmonella* (Haley et al., 2009). In 2005, an *E. coli* O157:H7 infection outbreak associated with iceberg lettuce occurred in Sweden, where 135 cases were reported. The cause of the outbreak was traced back to the river used for irrigation (Söderström et al., 2008). The recurrent multistate outbreak of *Salmonella* Newport linked to contaminated tomatoes was traced back to the shore of Virginia, where the strain was isolated from the pond water used for irrigation (Greene et al., 2008; Haley et al., 2009). Serrano and Jalapeno peppers multistate outbreak caused by the *Salmonella* St. Paul strain was traced and detected in an agricultural water sample collected in Mexico (Mody et al., 2011). An *E. coli* O157:H7 outbreak occurred in 2018 due to contaminated Romaine lettuce, once more with the outbreak strain detected in sediment collected from an agricultural water reservoir (Hoff et al., 2021).

To control produce contamination caused by preharvest irrigation water, several management and preventative approaches are being employed, which include modifying the irrigation method, reducing the amount of time produce is in contact with water, applying Good Agricultural Practices (GAP), and establishing physical barriers (Luna-Guevara et al., 2019). Disinfectant treatments using chemicals like chlorine, peroxyacetic acid or devices like UV treatments have been recommended when treating surface waters (Uyttendaele et al., 2015a). Chlorination is the most common and cost-effective method for treating wastewater. It is quite effective against enteric bacteria, but when the organic matter content is high in the water, chlorine reacts with it and releases harmful disinfection by-products (Veschetti et al., 2003).

Peroxyacetic acid has also demonstrated good activity in treating water and does not release toxic residues (Veschetti et al., 2003). An effective dosage of sanitizer can help mitigate microbial contamination in preharvest irrigation water, but effective dosing strategies must be determined.

CHAPTER 2

LITERATURE REVIEW

Prevalence of foodborne illness due to Preharvest Irrigation Water

Fresh produce includes fruits, vegetables, herbs, seeds, and nuts that can be whole, prepared (precut or reduced in size), ready to eat (no preparation required before consumption), and/or dressed (Yeni et al., 2016). Because eating fruits and vegetables is associated with leading a healthy lifestyle, various international organizations, such as the World Health Organization, encourage the daily intake of at least 400 g of fruit and vegetables per day (excluding potatoes and other starchy tubers) for the prevention of chronic diseases, such as heart disease, cancer, diabetes, and obesity (Callejón et al., 2015). Foodborne illness outbreaks or cases connected with fresh produce intake have increased significantly as a result of increased product consumption, globalization of the produce sector, and improved surveillance (Havelaar et al., 2010). Because fresh produce is frequently consumed in its raw state, with no processing step to eliminate harmful organisms, there is a risk of contamination with foodborne pathogens and, as a result, illness upon consumption (Carstens et al., 2019, Lynch et al., 2009).

In the past three decades, the incidence of foodborne illnesses that can be traced back to contaminated fresh produce has increased dramatically, making it a top priority concern in terms of food safety and public health (Gurtler & Gibson, 2022). Between 2004 and 2010, there were 1,779 foodborne outbreaks in the United States, with fresh produce accounting for 9.2% (163) of them (*National Outbreak Reporting System (NORS) Dashboard | CDC*, 2018). Foodborne diseases that are linked to fresh produce are most commonly caused by Gram-negative bacteria

such as *Salmonella enterica* subsp. *enterica* and Shiga toxin-producing *Escherichia coli* (STEC), as well as Gram-positive bacteria such as *Listeria monocytogenes* (Gurtler & Gibson, 2022).

When looking at the farm-to-fork chain, microbial contamination of fresh produce can occur at multiple steps, such as the cultivation of fresh produce, at harvest, during preparation and washing, within distribution chains and transport to retail operations, and even at the final step in the kitchen of the consumer (Matthews, 2013, Machado-Moreira et al., 2019). Preharvest hazards to produce have been identified as critical since it is difficult to disinfect produce once pathogen contamination has occurred in the field (Alegbeleye et al., 2018). Contaminated water sources with bacterial pathogens have emerged as potential contributors to fresh produce contamination, with the ability to contaminate a large amount of produce at once if sufficient populations exist in the source water (Faour-Klingbeil et al., 2016).

Irrigation is the application of water to the soil or plant during the agricultural production of farm produce to enhance the yield of fresh food when natural rainfall is insufficient for optimal production (Akinde et al., 2016). Because of the capacity of pathogens to live for extended periods within these two agrarian niches, irrigation water and agricultural soils are the principal reservoirs and transmission pathways of human pathogens at the preharvest stage (Jung et al., 2014). Irrigation water is a critical pathogen transmission route to farm produce as enteric pathogens from the soil, fecal materials, sewage overflow, and other sources can be introduced into the reservoirs, rivers, or lakes from which irrigation water is typically extracted (Rajwar et al., 2016). In the United States, under the Food Safety Modernization Act Standards for the Growing , Harvesting, Packing, and Holding of Produce for Human Consumption (Produce Safety Rule), requirements for water quality are established based on *E. coli*; for example, "no detectable generic *E. coli* in 100 mL of agricultural water" is a criterion that is described for

postharvest uses of agricultural water, whereas numerical criteria for agricultural water to be used directly on growing produce (except for sprouts) is based on a geometric mean of ≤126 CFU of *E. coli* per 100 mL of water and a statistical threshold of ≤410 CFU of *E. coli* in 100 mL of water (Banach & Van Der Fels-Klerx, 2020). *E. coli* concentrations in water are utilized as a hygiene indicator when monitoring water quality for agricultural applications, and it has also been found to be a suitable index organism for *S. enterica* and STEC (Ceuppens et al., 2015).

Most irrigation water in the world comes fromurface water or groundwater reserves such as aquifers (Uyttendaele et al., 2015b). The quality of irrigation water is usually determined by the source of the water, and the following irrigation water sources are listed in decreasing order of microbial hazard: untreated or inefficiently treated wastewater, surface waters, shallow groundwater, deep groundwater, potable water (Pachepsky et al., 2011). Agriculture in the United States is anticipated to utilize around 118 million gallons of water per day, with surface waterways accounting for more than half of total irrigation water (Gurtler & Gibson, 2022). Surface waters, such as open canals, ponds, lakes, rivers, and streams, are far more vulnerable to bacterial contamination than groundwater (Haley et al., 2009, Allende & Monaghan, 2015).

Surface watercourses can be contaminated by sewage discharges, septic tank pollution, storm drains, wild and domesticated animal excrement, run-off from contaminated areas, and industrial and municipal effluents (Steele & Odumeru, 2004). When surface water is utilized for irrigation, ditches, buffer strips, retention systems, and drainage systems can be built to prevent polluted water from draining into the surface water reservoir and by preventing potential overflow locations (Uyttendaele et al., 2015b). Sprinkler irrigation systems, canal irrigation (furrow irrigation), drip irrigation, hydroponic cultivation, and other irrigation methods can affect the transfer of contamination to crops because irrigation distribution networks can create

low-flow conditions in some parts of the network, which can contribute to the deterioration of water microbial quality (Uyttendaele et al., 2015b). Subsurface or drip irrigation, when compared to furrow and spray irrigation, reduces the danger of transmission to developing plants by decreasing the exposure of the irrigated water to the product unless they are a root crop (Oron et al., 2001).

S. enterica and STEC have caused the majority of foodborne outbreaks in fresh produce (Oron et al., 2001). A majority of outbreaks linked to irrigation water have been caused by *E. coli* O157:H7, which can resist harsh circumstances (Dirk Van Elsas et al., 2011b). In 2019, an *E. coli* outbreak connected to Romaine lettuce was reported, with 62 patients affected with the outbreak strain of *E. coli* O157:H7 in 16 states and the District of Columbia (Hoff et al., 2021). The FDA, in collaboration with the Centers for Disease Control and Prevention (CDC) and state partners, investigated farms and cooling facilities in California that were identified in traceback investigations (Hoff et al., 2021). The CDC analyzed water and sediment samples from an Adam Bros Inc. farm in Santa Barbara County, one of the farms identified in the traceback investigation (Hoff et al., 2021). The *E. coli* O157:H7 epidemic strain was discovered in silt within an agricultural water reservoir on the farm. The *E. coli* O157:H7 detected in the agricultural water reservoir was discovered to be genetically similar to the clinical isolates (Hoff et al., 2021).

In 2018, a multistate outbreak of *E. coli* O157:H7 infections linked to Romaine lettuce infected 210 people from 36 states, with 96 people hospitalized, including 27 people who developed a type of kidney failure called hemolytic uremic syndrome, and five deaths reported from Arkansas, California, Minnesota, and New York (Hoff et al., 2021). The FDA, CDC, and state partners began an environmental evaluation in the Yuma growing region, collecting

samples of water, soil, and manure (Hoff et al., 2021). CDC laboratory testing detected the outbreak strain of *E. coli* O157:H7 in water samples obtained from a Yuma growing zone canal. Once more, the *E. coli* O157:H7 detected in the canal water was genetically similar to the clinical isolates (Hoff et al., 2021).

A major outbreak of STEC occurred in Sweden in 2005, with 135 cases documented, and epidemiological investigations indicated iceberg lettuce as the most likely cause of the outbreak (Söderström et al., 2008). Polymerase chain reaction (PCR) was used in microbiological studies to identify Shiga-like toxin (Stx) (Söderström et al., 2008). After investigations, it was discovered that the lettuce was irrigated with water from a tiny stream, and water samples were positive for Stx_2 by PCR (Söderström et al., 2008). The same *E. coli* O157 Stx_2-positive strain was recovered from cases and livestock at a farm upstream of the irrigation site (Söderström et al., 2008).

According to the Centers for Disease Control and Prevention, *Salmonella* was responsible for around 53.4% of all foodborne illness outbreaks from 2006 to 2017, with fresh produce accounting for around 32.7% of these outbreaks (Sultana et al., 2018). The production of fruits and vegetables depends heavily on irrigation, and *Salmonella* contamination of irrigation water has been identified as one of the main causes of contaminated produce (Hanning et al., 2009). *Salmonella* has been found to exist and survive in surface waterways, including ponds, lakes, and rivers (Sultana et al., 2018). *Salmonella* was found in 79.2% of surface water samples tested in Georgia (Haley et al., 2009). Due to contaminated tomatoes produced on Virginia's eastern shore, a long-lasting multistate outbreak of *S.* Newport occurred (Greene et al., 2008). The 2002 outbreak led to 510 people with confirmed and suspected illnesses in 26 states, and the 2005 outbreak had many similarities (Greene et al., 2008). The traceback's findings revealed that the

tomatoes came from two growers/packing facilities on Virginia's Eastern shore (Greene et al., 2008). During this time, farms in this area exclusively sold their product to the Eastern and Central United States, consistent with the national distribution of cases of the *S.* Newport outbreak pattern (Greene et al., 2008). *S.* Newport was discovered in an irrigation pond water sample from a farm on Virginia's Eastern shore in October 2005 that matched the outbreak strain (Greene et al., 2008).

Salmonella Saintpaul-related Jalapeño and Serrano pepper outbreaks were traced back to farms in Tamaulipas and Nuevo León, Mexico, which sent peppers to the McAllen, Texas, distribution hub in 2008 (Klontz et al., 2010). At least 286 people were hospitalized due to this outbreak, which affected 1,442 people throughout 43 states, the District of Columbia, and Canada (Barton Behravesh et al., 2011). Jalapeno peppers used at Restaurant A during the case-patient exposure period were tracked down to Importer A in southern Texas during the traceback studies (Mody et al., 2011). Jalapeno peppers used in Restaurant Chain B restaurants throughout the case-patient exposure interval were tracked back to Importer B, located near Importer A (Mody et al., 2011). The outbreak strain was isolated from a jalapeno pepper sample received from Importer B (Mody et al., 2011). These peppers were tracked back to a packaging plant in Nuevo Leon, Mexico (Mody et al., 2011). The FDA investigated two Mexican farms (Farm A and Farm B) that were key suppliers of peppers to the packing plant during this period, while records show that other farms also supplied the packing facility during this period (Mody et al., 2011). Farm A produced Roma tomatoes as well as jalapeno and serrano peppers (Mody et al., 2011). *Salmonella* was found in environmental samples from Farm A, although none were serotype Saintpaul. Farm B, around 100 miles away from Farm A, was the packaging facility's primary pepper supply (Mody et al., 2011). It cultivated jalapeno and serrano peppers, but not

tomatoes (Mody et al., 2011). The outbreak strain was identified from two samples taken from Farm B: agricultural water and field-grown serrano peppers (Mody et al., 2011).

STEC

Within the family *Enterobacteriaceae, E. coli* is a Gram-negative, facultative anaerobe that is generally a commensal microorganism that, in the intestines of its human host, has a symbiotic and mutually beneficial connection with the human host (Tchaptchet & Hansen, 2011). *E. coli* is a harmless bacterium that is commonly used as an indicator organism for fecal contamination and hygiene breaches. Still, a number of different *E. coli* pathotypes have gained virulence factors, which have enabled them to adapt to new environments and, in certain circumstances, to cause disease (Farrokh et al., 2012). These virulence traits are usually encoded on genetic components that may be mobilized into new strains to form novel combinations of virulence factors or might be encoded on genetic elements that could previously have been mobile but have now evolved to become 'locked' within the genome (Kaper et al., 2004).

Some strains of *E. coli* are responsible for the spread of disease because they produce a toxin known as Shiga toxin and are referred to as "Shiga toxin-producing" *E. coli* or STEC (Farrokh et al., 2012). Bacteria known as enterohemorrhagic *E. coli* (EHEC) or verocytotoxic *E. coli* (VTEC) are related to the same broad group of bacteria (Farrokh et al., 2012). Enteropathogenic *E. coli* (EPEC), EHEC, enterotoxigenic *E. coli* (ETEC), enteroaggregative *E. coli* (EAEC), enter invasive *E. coli* (EIEC), and diffusely adherent *E. coli* (DAEC) are the six well-defined groups of intestinal pathogens (Nataro & Kaper, 1998). Infection with any one of these pathotypes can lead to three distinct clinical syndromes:

enteric/diarrheal illness, urinary tract infections (UTIs), and sepsis/meningitis (Nataro & Kaper, 1998).

STEC, are one of four *E. coli* pathotypes that are usually recognized as being the cause of diarrhea in humans caused by the consumption of contaminated food or drink, or through another fecal-oral route (Hunt, 2010). EHEC was classified as a subgroup of STEC, which were known to induce hemorrhagic colitis (HC) and hemolytic uraemic syndrome (HUS) in humans (Levine, 1987 Newell & La Ragione, 2018). STEC is distinct among *E coli* as it has and expresses the genes for Shiga toxins type 1 (Stx_1) and 2 (Stx_2) (Hunt, 2010). Most *E. coli* O157 isolates only generate Stx_2; Stx_1 and Stx_2 producers are occasionally identified but isolates generating Stx_1 exclusively are uncommon (Griffin & Tauxe, 1991). O157:H7 is the STEC serotype that is found the most frequently in the United States and more than one hundred STEC serotypes have been linked to human illness (Brooks et al., 2005).

Shiga toxin, for which STEC derive their name, is a toxin which is capable of killing host cells in the intestines and entering the bloodstream, where it can then harm other organs including the kidneys and the brain (Kintz et al., 2017). It is hypothesized that the organism colonizes the mucosa of the large bowel, which is characterized by an attaching and effacing (A/E) cytopathology, that is mediated by components of an approximately 43.4-kb pathogenicity island (PAI) called the locus of enterocyte effacement (LEE) (Nataro & Kaper, 1998). Resistance to stomach acidity is an important feature of STEC strains that may impact their ability to colonize the human gut, particularly at low infectious doses, as exposure to low pH induces an acid tolerance response, which has been shown to increase the survival of *E. coli* O157:H7 in mildly acidic foods (Leyer et al., 1995, Goodson & Rowbury, 1989).

Cattle, particularly ruminating post-weaning calves, and heifers, are thought to be the most significant reservoirs of STEC without symptomatic colonization among ruminants (Gyles, 2007). The phrase "super shedder" refers to cattle that have been found to shed excrement that has a high concentration of STEC (more than 10^4 CFU/g (Arthur et al., 2010). Despite the fact that STEC can be carried by ruminants, contamination of foods can occur as a result of the pathogen's release into the environment after being passed in ruminant feces (Erickson & Doyle, 2007).

The Centers for Disease Control and Prevention (CDC) estimates that there are 265,000 cases of STEC infections diagnosed annually in the United States, out of which STEC O157 is responsible for 36% of these diagnoses, meaning that non-O157 STEC infections account for 64% of all STEC diagnoses (Scallan, Hoekstra, et al., 2011). Food is the most often recognized carrier of STEC outbreaks in the United States, with 52% of *E. coli* O157:H7 infection outbreaks between 1982 and 2002 related to the intake of contaminated food (Rangel et al., 2005). The most prevalent item that is linked to STEC infections in the United States is ground beef that has not been properly cooked (Homas et al., 1995). Food was found to be responsible for 127 (64%) of foodborne outbreaks, with vegetable row crops (25%) being the most prevalent culprit, followed by beef (20%), dairy (15%), and fruit (6%) (Tack et al., 2021). Of the vegetable row crop outbreaks, thirty of the 32 were related to leafy greens, with Romaine lettuce attributed to seven outbreaks and spinach being the most common culprit (Tack et al., 2021). STEC is highly suited to thrive in animal feces water containing animal feces and soil, which has resulted in the contamination of fresh food when manure contaminates irrigation water or is used for fertilization (Erickson & Doyle, 2007, Islam et al., 2004).

This pathogen is resistant and can live for long periods in various environments, including water and soil, temperatures as low as freezing and as high as refrigeration, as well as acidic and dry conditions (Islam et al., 2004). A physiologically stressed STEC may be particularly troublesome since cells in this state might be difficult to recover and identify (Baker et al., 2016). Viable but nonculturable cells (VBNC) are problematic because they cannot be identified on standard culture medium and can lead to incorrect assumptions about the real number of cells in an environment (Baker et al., 2016).

Salmonella enterica

Salmonella is a Gram-negative, rod-shaped facultative anaerobic bacteria that cause internal infections in people and animals (Crum-Cianfl One, 2008, Silbergleit et al., 2020, Andino & Hanning, 2015). It is typically 2-5 microns in length and 0.5-1.5 microns broad, and they move via peritrichous flagella (Andino & Hanning, 2015). *Salmonella* is a member of the Enterobacteriaceae family and consists of two species, *S. bongori* and *S. enterica* (Andino & Hanning, 2015, Levantesi et al., 2011). *S. enterica* is the only species which causes human illness (Crum-Cianfl One, 2008) The species *S. enterica* is further subdivided into six subspecies (*enterica, salamae, arizonae, diarizonae, indica,* and *houtenae),* of which the *S. enterica subspecies enterica is primarily* linked with humans and other warm-blooded animals (Levantesi et al., 2011). *Salmonella* infections manifest as a disease called salmonellosis causing a gastrointestinal illness and fever (Hurley et al., 2014). When salmonellosis becomes systemic, intestinal fevers can develop following diarrhea (Kurtz et al., 2017). When *S.* Typhi is the causative organism, enteric fever is a frequent sign that is marked by temperature, anorexia, headache, fatigue, myalgia, constipation, and other non-specific symptoms (Ehuwa et al., 2021).

Salmonella is estimated to cause approximately 1.35 million infections, 26,500 hospitalizations, and 420 fatalities in the United States each year, with food being the source of the majority of these diseases (Lee et al., 2021). Salmonella infection typically causes symptoms 12 to 72 hours after exposure and lasts four to seven days (Rahman, 2015). The intensity of salmonellosis is determined by the particular serotype accountable for the infection and the host's health state (Ehuwa et al., 2021). *Salmonella* contamination is most commonly linked with chickens, livestock, and their feeds, but dried foods, baby formula, fruit and vegetable products, and pets have also become significant (Ehuwa et al., 2021). While water is a prevalent mode of infection for typhoidal *Salmonella* serovars, non-typhoidal *Salmonella* is primarily recognized as foodborne pathogens (Levantesi et al., 2011).

Within the *Salmonella* genus, *Salmonella enterica* is further subdivided into six subspecies with at least 2500 serotypes distinguished by variations in O (somatic) and H (flagellar) antigens, and approximately 99% of *Salmonella* strains that cause infection in humans or other mammals belong to the *Salmonella enterica* species (Kurtz et al., 2017). More than 80% of outbreaks caused by serotypes Enteritidis, Heidelberg, and Hadar were linked to eggs or poultry. In contrast, plant items were linked to more than 50% of outbreaks caused by serotypes Javiana, Litchfield, Mbandaka, Muenchen, Poona, and Senftenberg (Jackson et al., 2013). Typhimurium and Newport, two of the most prevalent *Salmonella* serotypes, had a broader variety of implicated food items (Bowen et al., 2006, Jackson et al., 2013). Serotype Typhimurium has a well-characterized ability to infect different species and can survive in the environment for an extended time; these two factors contribute to this serotype's ability to be one of the most prevalent causes of salmonellosis in the United States (Baudart et al., 2000, Rabsch et al., 2002). *S.* Typhimurium ranks second in Europe and third in the United States in human

salmonellosis cases (Ferrari et al., 2019). *S.* Thompson has been found as the source of foodborne epidemics linked to the intake of cilantro, arugula, poultry, meat, egg, bread, and smoked salmon (Campbell et al., 2001).

Irrigation water is a pathway of crop contamination in produce-related *Salmonella* outbreaks (Scallan, Griffin, et al., 2011, Levantesi et al., 2012, Thunberg et al., 2002). Furthermore, some *S. enterica* strains can attach to plant surfaces, where they can live for extended periods with the opportunistic capacity for growth based on environmental conditions (Ma et al., 2011, Kurtz et al., 2017). In research performed by Jackson et al. (2013), serotypes Newport (30%) and Javiana (20%) caused 50% of the ten leafy vegetable-associated outbreaks (Jackson et al., 2013). Lapidot and Yaron (2009) showed the capacity of *S.* Typhimurium to move from infected irrigation water in plant roots to edible sections of mature parsley, but only in extremely contaminated irrigation water (7.6 log CFU/mL) (Lapidot & Yaron, 2009). Poona infections linked to cantaloupe intake prompted investigators to assume that melons were indirectly contaminated via packing tools or wash water polluted by reptiles (Bowen et al., 2006). Non-Typhimurium serotypes caused the majority of fresh produce outbreaks associated with fruits, veggies, and sprouts in Australia, with *S.* Saintpaul, Litchfield, and Oranienburg causing more than half of the outbreaks associated with these foods (Ford et al., 2018).

More than 30 *Salmonella* effectors have been investigated and demonstrated to perform key virulence functions in animal cells by influencing various host cell functions (McGhie et al., 2009). *Salmonella* genetic equipment, previously believed to be animal-infection specific, now plays a significant part in the infection of both animals and plants (Schikora et al., 2012). *Salmonella* has two pathogenicity islands, SPI-1, and SPI-2, which encode Type III secretion systems (T3SS-1 and T3SS-2) and a set of effectors where T3SS are essential virulence factors

for many Gram-negative pathogens (García et al., 2014, Ibarra et al., 2009). T3SS-1 is synthesized during the extracellular stage of animal infection, whereas T3SS-2 is activated after entry into animal cells (García et al., 2014). These systems are made up of a specialized secretion apparatus that transports effector proteins from the bacterial cytoplasm into the host cell, and the translocated T3SS effector proteins successfully enable bacteria to 'hijack' many important intracellular processes (Ibarra et al., 2009).

The plant environment affects *Salmonella* pathogenicity islands, T3SS, and effectors (García et al., 2014). SPI-1 genes are known to be triggered by high osmolarity, low oxygen levels, and short chain fatty acids, and it was recently demonstrated using a promoter-reporter fusion that the SPI-1 gene *prgH* is expressed when in touch with Arabidopsis root cells (Hautefort et al., 2003, García et al., 2014). Kroupitski et al. (2009) found that for *Salmonella* to penetrate the core of lettuce leaves, motility and the capacity for chemotaxis are necessary (Kroupitski et al., 2009). *Salmonella* motility is accomplished using 5-10 peritrichous or haphazardly distributed filamentous flagella stretching from the cell surface (Ibarra et al., n.d.). *S.* Typhimurium has two loci that encode distinct flagellins, *fliC* and *fljB*, and their expression is controlled by a switch mechanism that allows only one flagellin to be expressed at a time (McQuiston et al., 2008).

Salmonella can easily cope with extreme environmental circumstances such as temperatures that are lower or higher than optimum, pH values, or desiccation (Podolak et al., 2010). *Salmonella* can live in a wide range of pH (4.05-9.5) environments and proliferate in a wide range of temperatures (7-48°C) (Fatica & Schneider, 2011). *Salmonella* has been found to persist in low-moisture food items for prolonged periods of time (Podolak et al., 2010). *Salmonella* heat resistance rises with decreasing moisture and is influenced by a variety of

variables, including the strain and serotypes evaluated, prior growth and storing circumstances, physical and chemical food makeup, test media, and the media used to restore heat-damaged cells (Podolak et al., 2010). *Salmonella* has been found to create biofilm-like structures on the surface of roots, preferring colonizing areas near emerging lateral roots and injured tissues (Leonardo Iniguez et al., 2005). Biofilm formation in *Salmonella* is regarded as one of the virulence tools that gives the bacterium a survival edge (Fàbrega & Vila, 2013). Biofilm's extracellular matrix includes polysaccharide layers that shield the microbes from their harsh surroundings (Rana et al., 2021). Quorum sensing has been shown to enhance motility in *S.* Typhimurium by triggering the expression of genes involved in flagella construction (da Conceição et al., 2015). The *QseC* quorum-sensing sensor kinase has also been discovered to play a part in this pathogen's motility and swine colonization (Rana et al., 2021).

Disinfection of Preharvest Agricultural Water

Disinfection is the method of inactivating or eliminating pathogenic microorganisms from agricultural water systems (Mishra et al., 2018). Disinfection is carried out in the event of a known health threat or to mitigate potential foodborne pathogens (Collivignarelli et al., 2018). Highly pathogenic microorganisms in the water represent a significant hazard to public water safety and the natural environment (S. Zhang et al., 2022). Disinfection treatment is a critical step to ensure and maintain microbiological quality (Somani et al., 2011). Even though water quality management is often considered a time-consuming and costly activity, it is suggested that it should be highly prioritized when known microbiological hazards exist at a sufficient concentration that they are likely to contaminate crops (Suslow, 2001). The goal of water treatment should be to use a minimal dosage of effective sanitizer for microbial disinfection,

which is commonly described as the product of Concentration (C) and Time of exposure (t), or $(C \times t)$ (Suslow, 2001). Growers and other risk managers should have evidence-based studies verifying the effectiveness of any water treatment (Krishnan et al., 2021. Effective pathogenic bacterial control depends on the correct use of disinfectants and a reliable dosing system (S. Zhang et al., 2022).

Disinfection is part of a comprehensive sanitation and safety management program. It can be accomplished by applying disinfectants or sanitizers such as hydrogen peroxide, chlorine dioxide, sodium, and calcium hypochlorite or through physical processes like microfiltration or UV light (Mishra et al., 2018). For the inline treatment of preharvest agricultural water, a variety of methods, including chemigation with peracetic acid (PAA) or chlorine-based compounds and treatment with devices such as ultraviolet light (UV), are being proposed based upon their suitability for deployment in the field setting (Krishnan et al., 2023.

Because of their low cost and excellent disinfection efficacy, chlorine-based disinfectants have become the most widely used (Zhang S. et al., 2022). But in a study conducted by Ragazzo et al., PAA showed equivalent inactivation of *Escherichia coli* when compared to hypochlorites (Ragazzo et al., 2020). PAA requires extremely high concentrations to be effective as a virucide, whereas chlorine, both free and mixed, is a powerful bactericide but looks to be less effective as a virucide (Rudd & Hopkinson, 1989). Temperature, pH, and suspended particles are the water quality indicators that can speed up disinfectant degradation (Luukkonen & Pehkonen, 2017).

Other interferences like inorganic substances such as ferrous and manganese ions, nitrites, sulfides, and organic matter also affect the disinfection efficiency by reducing the concentration of oxidizing disinfectants and causing a reduction in microorganisms' inactivation (Collivignarelli et al., 2018). Overdosing or misuse of these chemical disinfectants can cause

their reaction with organic and inorganic precursors resulting in the production of disinfection by-products (DBPs) with negative health consequences (Collivignarelli et al., 2018). As the water quality can vary between different sources, treatment efficacy should be studied thoroughly as the chemical disinfection efficiency can vary based on differences in water quality (Bester & Lamprecht, 2015).

Chlorine

Chlorination is a chemical treatment for eliminating the growth of microorganisms (Migliaccio et al., 2009). Chlorination is typically efficient, cost-effective, and user-friendly and may be used in any operation (Mishra et al., 2018). Because of these advantages, chlorination has played an important role in reducing waterborne infections for almost a century. Chlorination, considered to be the first chemical water disinfection process, was started to be used as a standard unit process at water treatment plants in 1902, and by the early 1940s, chlorination had become the recognized treatment method globally, with more than 85% of drinking waters supplies in the United States chlorinated (Gray, 2014). By the end of the 1960s, people interpreted the presence of chlorine in drinking water as an indication of water safety (Gray, 2014). Chlorine (Cl) is soluble in water, with high disinfection rates and powerful oxidizing properties (Mishra et al., 2018). Chlorine has been used primarily to inactivate or eliminate pathogenic bacteria, fungi, viruses, cysts, and other microorganisms related to seed, cuttings, irrigation water, contact surfaces, and human contact with fresh produce during preharvest, harvesting, and handling operations as well as to prevent their transfer from one item to another (Mishra et al., 2018). Chlorine is a combination of chlorine gas (Cl_2), hypochlorous acid (HOCl), and hypochlorite ions (OCl^{-}) in varying proportions depending on the pH of the

water (Mishra et al., 2018). The terms free chlorine, reactive chlorine, and available chlorine all refer to the quantity of chlorine that is available for oxidative reaction and disinfection (Mishra et al., 2018). Chlorine-based treatments are more successful in earlier research due to their residual activity, which provides ongoing efficacy over time, but the disinfection byproducts can be harmful to human health when additional inputs like herbicides and insecticides are used (Liang et al., 2009).

Chlorine's basic interaction with water creates hypochlorous acid (HOCl) and hydrochloric acid (HCl), the most efficient form of free chlorine residual in chlorination disinfection for controlling bacterial growth (Migliaccio et al., 2009). The amount of hypochlorous acid (HOCl) released in the water after treatment is determined by the pH of the water, the amount of organic material in the water, and, to a lesser degree, the water temperature Suslow, 2001). Depending on the pH, hypochlorous acid can further degrade into a hydrogen ion and a hypochlorite ion, both of which are ineffective disinfectants (Chlorine Health Hazard Information Sheet. In *FSIS Environmental Safety and Health Group*. Retrieved, June 7, 2023). At lower pH levels (more acidic circumstances), the amount of HOCl present in the solution and hence active will be greater (Migliaccio et al., 2009). To guarantee enough HOCl activity without the generation of chlorine gas, which can lead to health hazards for employees and increased corrosion on equipment, the solution should be kept between pH values of 6.5 and 7.5 (Mishra et al., 2018, Suslow, 2001). The active species in germicidal activity is HOCl, and the concentration of OCl^- is an important determinant in determining antimicrobial effectiveness (Fukuzaki, 2006).

HOCl is a fast-acting and potent antimicrobial agent that is easily transferred across a microbial cell to initiate the killing process and further interacts with several biomolecules,

including sulfur-containing amino acids, lipids, nucleic acids, and membrane components, causing severe cellular damage (Nizer et al., 2020, Suslow, 2001). Because of its net neutral charge and small molecular size, HOCl can pass through the lipid bilayer of the plasma membrane by passive diffusion (Fukuzaki, 2006). As a result, HOCl may attack the microbial cell not only from the outside but also from within, speeding inactivation and increasing germicidal action (Fukuzaki, 2006). Chlorine's major impact is to change the chemical structure of the enzymes that are the foundation of bacteria's feeding systems, inactivating them, and so preventing their development and existence (Collivignarelli et al., 2018). Although the mechanism of HOCl or -OCl germicidal effect is not entirely understood, it is thought to be owing to the suppression of required enzyme activity for development, damage to the membrane and DNA, and potential impairment to membrane transport ability (Fukuzaki, 2006). In clean water, relatively low amounts of HOCl (about 1-2 parts per million) for 1 minute contact time are adequate to destroy most bacteria and viruses (Suslow, 2001). Contact time or concentration must be increased when water quality and complexity diminish (Suslow, 2001).

At pH levels less than 4, Cl_2 becomes the dominating chlorine specie, whereas at higher pH values (between 8.5 and 10), the concentration of HOCl drops near to zero and -OCl becomes the principal component of the solution (Fukuzaki, 2006). Because a higher pH promotes the creation of more hypochlorite ions and resulting in less hypochlorous acid in the water, disinfection is more effective at a lower pH rather than a higher pH (FSIS Environmental Safety and Health Group, June 7, 2023). Below pH 6.0, toxic chlorine gas (Cl_2) is generated, which is ineffective as a water disinfectant (Suslow, 2001). The addition of organic matter increased COD from 250 mg L^{-1} to 1000 mg L^{-1}, resulting in a significant rise in chlorine demand (Hassenberg et al., 2017). According to George Weber & Colonel Max Levine (1944), the

antimicrobial activity of chlorine rises with increasing temperature (George Weber & Colonel Max Levine, 1944). In a study by Hassenberg (2017), decreasing the temperature of the processing water (from 15°C to 2°C) resulted in a reduced and delayed reduction of *E. coli* counts, where at a ClO_2 concentration of 3 mg L-1, microbes were significantly and completely inactivated at 15°C after 0.5 min, but only partially reduced at 2°C after 1 min (Hassenberg et al., 2017). However, in research done by (Sisti (1998), free chlorine was able to reduce the number of surviving bacteria by one or more log10 at lower temperatures when compared to the findings obtained with the same dosage at 20° (Sisti et al., 1998). This variation might be explained by the fact that free chlorine is inactivated/combined more quickly with compounds with lower microbicidal activity at 20°C (Sisti et al., 1998).

Because of its ease of use and low cost, hypochlorite is a popular water disinfectant in the produce business (Suslow, 2001). When chlorine in any form is added to water, it immediately starts reacting with the other chemicals and materials in the water as well as with the water itself, which may result in some desirable reactions producing a residual chlorine solution (such as hypochlorous acid) capable of killing microorganisms (FSIS Environmental Safety and Health Group, June 7, 2023). Excessive treatment, particularly hyperchlorination (the use of high levels of chlorine), has a number of known and potential negative effects on product sensory quality, the environment, and human health, and may result in the production of excessive amounts of harmful disinfection by-products (DBPs) in the water (Suslow, 2021; Van Haute et al., 2013). Halogen compounds like THMs, haloacetic acids (HAAs), chlorophenols, chloral hydrate, and haloacetonitriles combine easily with organic components in water to create disinfection by-products (Chang et al., 2000; Collivignarelli et al., 2018; Monarca et al., 2002)

Peracetic Acid

PAA, CH_3COOOH, is the peroxide of acetic acid (Koivunen & Heinonen-Tanski, 2005). It is a clear, colorless liquid with an acidic pH of less than 2, a strong, pungent, vinegar-like odor, and no foaming capabilities (Dery et al., 2021, Kitis, 2004). When hydrogen peroxide (H_2O_2) and acetic acid (CH_3COOH) mix, PAA is formed, which when decomposed yields acetic acid and oxygen (Collivignarelli et al., 2018). PAA combines the reactive oxygen properties of peroxide within an acetic acid molecule and belongs to the family of organic peroxides, which are man-made compounds (Kitis, 2004). Because of the chemical equilibrium between its components, commercially marketed PAA-based products identify both PAA and H_2O_2 as active ingredients. (Ghostlaw et al., 2020). Many solutions on the market have variable ratios of H_2O_2 to PAA, making it difficult to pick an efficient sanitizer for commercial usage. (Ghostlaw et al., 2020). Commercially accessible PAA solutions (10% to 15%) utilized in the industry are substantially more stable than higher- and lower-strength solutions (Kitis, 2004). It is commonly used in irrigation water treatment at concentrations ranging from 5 to 10 ppm (Dery et al., 2021). PAA is a powerful oxidant and disinfectant with a higher oxidation potential than chlorine or chlorine dioxide. (Kitis, 2004). The breakdown products of PAA are acetic acid, hydrogen peroxide, oxygen, and water, and they are non-toxic and do not generate secondary pollution (Chang et al., 2000). In a study by Zhang et al. (2022), PAA was found to be a superior disinfectant to sodium hypochlorite in tropical and warm-temperature settings (S. Zhang et al., 2022).

Since PAA is a powerful oxidizer and disinfectant, it has also shown a wide range of bactericidal properties (S. Zhang et al., 2022). In general, the disinfection efficacy of PAA against microorganisms can be graded as follows: bacteria>viruses>bacterial spores>protozoan

cysts (Kitis, 2004). Peracetic acid demonstrated remarkable antibacterial activity, particularly under acidic circumstances, lowering vegetative bacterial populations by a factor of log 6 in 1 minute at 25°C using a solution containing 1.3 mmol/l of peracetic acid. (Baldry, 1983). When PAA is introduced to water, reactive oxygen is released, which is responsible for the oxidation process and disinfection effects (Dery et al., 2021). The PAA disinfection method involves the direct oxidization, or loss of electrons, of bacterial cell walls (Dery et al., 2021). PAA emits reactive oxygen or generates reactive hydroxyl radicals that assault the bacterial cell, destroying the cell wall and membrane as well as specific enzymes and DNA (Kitis, 2004). When electrons are lost from the cell wall, interactions between enzymes and proteins break down, causing the cell structure to be disrupted. (Dery et al., 2021). Sensitive sulfhydryl and sulfur linkages in proteins, enzymes, and other metabolites are prone to be oxidized, as are double bonds (Kitis, 2004). Its antibacterial activity is most likely due to thiol group oxidation in proteins and membrane disruption (Russell, 2003). As the cell wall and membrane continue to disintegrate, cellular functions cease, intracellular components seep out and are further damaged, and cell death ensues (Nguyen et al., 2014). Commercially available PAA frequently contains significant levels of H_2O_2, which also has antibacterial capabilities, albeit these are overshadowed by PAA disinfection strength (Vandekinderen et al., 2009).

When compared to sodium hypochlorite, peracetic acid offers several benefits. One of the numerous benefits of peracetic acid is that its breakdown creates just acetic acid and oxygen, therefore it has no effect on the finished product or the waste treatment process (Kunigk et al., 2001). There were no halogen-containing byproducts discovered in PAA-treated water, while many halogen-containing byproducts were found in water disinfected with ClO_2 or NaClO which is one of the most significant benefits of PAA over other commonly used chemical disinfectants

(Monarca et al., 2002). Under biochemical standard state conditions (pH 7.0, 25 °C, 101.325 Pa), the oxidation-reduction (redox) potential of PAA approaches that of free chlorine, suggesting that acid peracetic and free chlorine may have similar efficiencies in preventing planktonic bacteria regrowth in the absence of organic matter (Carrascosa et al., 2021). PAA is also resistant to peroxidases and keeps its action better in the presence of organic loads or food residues than chlorine throughout a wide temperature range (Small et al., 2007, Hilgren et al., 2007).

PAA may successfully kill a wide range of bacteria, but its disinfection impact is frequently influenced by environmental conditions such as pH, temperature, turbidity, and organic matter in water, and some contaminants in water (Kitis, 2004; Zhang et al., 2020). It is efficient at pH values ranging from 3.0 to 7.5, in contrast to chlorine compounds, which emit chlorine gas at a pH of 6.0 and lose efficiency above 8.0 (Kunigk et al., 2001, Kunigk & Almeida, 2001). In a study by Ghostlaw et al. (2020), PAA was more stable in pH 5 and 6.5 samples than in pH 9 samples (Ghostlaw et al., 2020). Higher pH values decreased the efficacy and stability of H_2O_2 and PAA, but lower pH values (below the pKa of PAA) enhanced lethality to *E. coli* O157:H7 and sanitizer stability (Ghostlaw et al., 2020). Temperature changes in processing water can occur when field heat from the produce transfers to the water, influencing sanitizer stability and efficacy over time (Ghostlaw et al., 2020). Lower temperatures during treatment enhanced sanitizer stability, but at the expense of microbial inactivation rate (Ghostlaw et al., 2020). Although the temperature has an effect on antimicrobial efficacy, PAA is still effective at low temperatures (Stampi et al., 2001).

When compared to chlorination, using modest dosages of PAA gives benefits in terms of producing minor amounts of disinfection byproducts while also enhancing the microbiological quality of water (Bonetta et al., 2021). In a study by Krishnan et al. (2023), at concentrations of 3

and 5 ppm, PAA was more effective than sodium hypochlorite in inactivating bacteria for both surface and groundwater sources but at 7 ppm, the effectiveness of PAA and sodium hypochlorite showed no difference for both surface and groundwater because, at 7 ppm, enough free chlorine in the undissociated form was created to produce results comparable to PAA at the same concentration (Krishnan et al., 2023). Increased sanitizer concentration allowed for greater residual PAA to lower bacterial counts over time and after re-inoculation (Ghostlaw et al., 2020).

Although chlorine-based disinfectants are widely employed in wastewater treatment facilities, their halogen compounds are much more easily formed as disinfection by-products with organic molecules in water than PAA (Stampi et al., 2001). However, because PAA disinfection is substantially slower, a greater disinfectant residual is required to achieve similarly quick microbiological inactivation (Carrascosa et al., 2021). The disadvantages of using PAA include its instability at concentrations above 15% and its higher cost as compared to conventionally used sanitizers (Kunigk & Almeida, 2001).

With the possibility for very low by-product creation, adopting PAA- based combination treatments are a promising alternative to using chlorine and its combination treatments, which often exhibit byproduct formation (chlorine concentrations as low as 1 ppm (Santoro et al., 2007). PAA and PAA + UV therapy resulted in a substantial drop in pH ($p<0.05$) (Krishnan et al., 2021). The use of a chemical disinfectant such as PAA in conjunction with UV irradiation has the potential to enhance disinfection by a variety of processes, including direct nucleic acid destruction and the creation of extra free radicals, resulting in oxidative stress (Sun et al., 2018).

Because PAA has good antimicrobial properties at low temperatures in the pH range of 3-7.5 and the disinfection by-products produced by PAA were mostly non-mutagenic and a short

contact time dependence, it can be used as a disinfectant in the food industry, replacing the traditionally used hypochlorite (Kitis, 2004, Monarca et al., 2002).

Conclusion

Currently, there are no registered antimicrobial treatments and protocols that can be authorized for disinfecting irrigation water systems (FDA, 2020). There are various external factors that can affect the efficacy of the sanitizers used to disinfect agriculture waters. This study will help in evaluating foodborne pathogens (Shiga-toxin producing *E.coli* and *Salmonella enterica*) in the preharvest irrigation water system to assess the performance of commercially available agriculture water sanitizers currently used in the industry and provide recommendations of treatment efficacy. Our research may ultimately help in establishing science-based recommendations for treatments which are able to inactivate *Salmonella* or STEC in surface water which may come in contact with fruits or vegetables during preharvest activities in Georgia operations. This can be a valuable step towards the safety of fresh produce and can protect consumers from foodborne illnesses when the quality of surface water is not deemed to be of safe and adequate sanitary quality.

CHAPTER 3

MATERIALS AND METHODS

Introduction

This research focused on the reduction of the test microorganisms by working with two EPA-registered sanitizers: free chlorine obtained from calcium hypochlorite tablets (Accu-Tab Chlorination systems, Houston, Texas) and peroxyacetic acid solution (SaniDate® 12.0, Biosafe Systems LLC, 22 Meadow St, East Hartford, CT). Free chlorine was tested at 4 and 10 ppm, and PAA was tested at a concentration of 6 and 10 ppm. The sanitizers were evaluated for a contact period of 5 and 10 min at 12 ℃ and 32 ℃.

Experimental design. Table 1 lists experimental parameters that were evaluated in two surface water sources in Georgia used for the production of fruits and vegetables for a total of 32 treatment combinations. All the treatment combinations were performed in three technical replicates and two biological replicates, with six samples analyzed per treatment combination.

Collection And Characterization of Agricultural Water

Forty-liter water samples were aseptically collected from two different locations on farms that use them for irrigation located in Tifton, GA, USA. The water was collected in containers from the middle of the pond at 0.5m depth to avoid any sediment or overly turbid water. All the collected samples were transported back in the ice coolers to the Food Science Building at the University of Georgia, Athens, GA.

The physicochemical parameters, pH, water temperature, turbidity, dissolved oxygen, conductivity, and air temperature were analyzed. COD was determined using a high-range COD vial (HACH digestion solution for 20-1500mg/L range, Hach Company, Loveland, CO), dry thermostat digital reactor (HACH DRB2000, Hach Company), and a multiparameter portable

colorimeter (HACH DR900, Hach Company). A portable multi-parameter meter (HACH HQ4300, Hach Company, Loveland, CO) was used to measure the ORP, pH, temperature, and conductivity. Turbidity was determined using the multiparameter portable colorimeter (HACH DR900, Hach Company).

Water was then stored at -18°C until used for experiments. All samples were thawed to room temperature prior to use.

Test Microorganisms

Both Shiga-toxigenic *Escherichia coli* and *Salmonella enterica* isolates were evaluated as cocktails separately. Table 2 indicates the ATCC numbers of the test microorganism strains used in this study. These strains were made rifampicin-resistant by growing them in the presence of rifampicin to a concentration of 80 µg/mL (Thermo Fischer Scientific, 1 Reagent Ln, Fair Lawn, NJ). This was done to allow selective growth of these microorganisms and eliminate any background microflora.

Preparation of Test Microorganism

The bacterial stock strains were stored at -80°C. A 10 µl aliquot of each individual stock strain was streaked and grown in duplicates on rifampicin-resistant Brain Heart Infusion agar plates (rBHI Agar, BD Difco, Pittsburgh, PA) and were incubated at 35 °C for 24 h. The following day, a colony was taken from each streaked strain. This inoculum was transferred into the rifampicin-resistant Brain Heart Infusion Broth (rBHI; Thermo Scientific, 300 Industry Drive Pittsburgh, PA) and incubated at 35 °C for 24 h. After 24 h of incubation, 1 mL of the culture was transferred into six Eppendorf tubes and centrifuged at 8000 X g for 3 min. The supernatant was discarded, and the pellet obtained in each Eppendorf tube was diluted by resuspending it in Butterfield's Phosphate Buffer Diluent Water (PBDW; MFG, Hamilton, NJ). To prepare the

bacterial cocktail, equal volumes of the individual strains of the test microorganisms were combined. To determine the initial concentration of the bacterial cocktail, the mixed culture was serially diluted and spiral plated onto rBHI in duplicate and incubated at 35°C for 24h.

Agricultural Water Inoculation

Each test organism cocktail included the mixed culture of STEC or *S. enterica*. For each experimental run, flasks were prepared in triplicate sets for each treatment combination as described in Table 1. Agriculture water (98 mL) was added to each of the sterilized flasks. The flasks were inoculated by adding a 1mL aliquot of the inoculum cocktail. The flasks were swirled to mix the bacterial cocktail in the water samples and then equilibrated at the given temperatures for at least 30 min.

Sanitizer and neutralizer Preparation

In the meantime, sanitizer stock solutions were prepared in a liter of Phosphate Buffer Diluent Water. For chlorine, 1.1-1.2g and 2.7-2.9g of Accu-Tab Tablets were added to obtain 4ppm and 10ppm, respectively in the agriculture water sample. For PAA, 5-5.2 mL and 8-8.3 mL of SaniDate® 12.0 were added in order to reach 6 ppm and 10 ppm respectively in 100 mL of the agriculture water sample. The neutralizer was prepared by dissolving 28g of 97% Granular Sodium Metabisulfite (Pittsburgh, PA) in a liter of PBDW. The concentration of the sanitizer and functioning of the neutralizer in agricultural water was determined by using the FAS-DPD Chlorine/Bromine test kit (LaMotte, Chestertown, MD) and Low Range Peracetic Acid Test Kit (AquaPhoenix Scientific, Hanover, PA) for free chlorine and PAA, respectively.

Sanitizer Exposure and Neutralization

Aliquots of the prepared sanitizer concentrations (1 mL) were added to the equilibrated flasks and exposed to the inoculated agriculture water for the contact period specified. For the

control treatment, PBDW was added instead of sanitizers. The flask was swirled in intervals during the contact period in order to mix all the contents in it. Following the contact period, the SMBS neutralizer solution (1 mL) was added to the sanitized agricultural water flasks to neutralize its contents.

Bacterial enumeration- Serial dilution of the neutralized material was performed by considering the dilution factor of the flask as 10^0. The dilutions were plated in quadruplicate using a spiral plater, which deposited 100 µl total volume (Eddy Jet 2W Spiral Plater, NY, USA). The prepared plates were incubated at 35 °C for 24 h. The bacterial growth was analyzed using Automated Colony Counter - SphereFlash®(Neutec Group Inc, Farmingdale, NY).

Sterility Controls

To test the sterility of the neutralizer, sanitizer, agriculture water, and PBDW, 1 mL of each was plated in duplicate. This sterility control test confirmed that only the rifampicin-resistant strains prepared were growing and eliminated any doubt of contamination. The acceptance criteria for these study controls were a lack of growth.

Neutralization Confirmation Tests

The below-mentioned set of tests was performed to confirm the neutralizer activity. In the Test Culture Titer (TCT), 0.1 mL of the bacterial cocktail was added to 10 mL of PBDW, mixed by vortexing, and plated after waiting at least two minutes. The acceptance criterion for this study control was the growth of bacteria. In the Neutralization Confirmation Treatment (NCT), 1 mL of the sanitizer of a given concentration was added to 9 mL of the neutralizer and mixed by vortexing. After 30 seconds, 0.1 mL of the cocktail was added. This was further vortexed and plated after holding the mix for at least two minutes. The NCT bacterial population was expected to be within 1 log of the TCT. In the Neutralizer Toxicity Treatment (NTT), 0.1

mL of the bacterial cocktail was added to 10 mL of the neutralizer, mixed by vortexing, and plated after waiting at least two minutes. The acceptance criterion for this study control was the growth of bacteria within 1 log of the TCT.

Data Analysis

Treatments for both STEC and *S. enterica* were performed independently in three technical and two biological replicates (n=32). JMP Pro 16.0.0 Software (SAS Campus Drive Cary, NC) was used to conduct Analysis of Variance (ANOVA) on single effects and mean separation with Tukey's honestly significant differences (HSD) Test was used to analyze the two-way interaction between the significant effects to compare Log CFU/mL reduction in STEC and *S. enterica* among different sanitizer concentrations considering contact time and temperature as significant effects.

Factors	Levels
Chemical	Chlorine (4 ppm and 10 ppm) PAA (6 ppm and 10 ppm)
Exposure time	5 and 10 min
Water source	Source 1 and Source 2
Temperature	32°C and 12°C

Table 1. Experimental parameters evaluated.

Shiga- Toxin producing *E. coli*	***Salmonella enterica***
43895	BAA-3140
BAA- 2196	BAA-3142
BAA-2215	BAA-3140
BAA-2192	BAA-3141
BAA-2440	BAA-3137
BAA-2219	BAA-3136
BAA-2193	BAA-3139

Table 2. ATCC numbers of the test microorganism strains.

CHAPTER 4

RESULTS

STEC

Sanitizer treatment, agriculture water sources, contact time, and temperature were considered as fixed effects as each of these factors played a significant role and their two-way interactions had a significant effect ($p<0.0001$) on the log reduction of STEC. Inactivation of STEC depended on sanitizer treatment the most, and the interaction of temperature, contact time, and sanitizer concentration had the least influence on STEC reduction ($p>0.05$). Compared to their respective controls, each sanitizer treatment combination caused at least a 5 log reduction of STEC in all the interactions of interest (Fig. 1 and Fig. 2).

Sanitizer Effectiveness

PAA and chlorine at higher concentrations had the highest efficiency in reducing STEC and caused a 6.12- 6.18 log CFU/mL log reduction. No significant difference was observed between the chlorine and PAA at the higher and lower concentration ($p>0.05$), demonstrating that each chemistry was equally effective in the inactivation of STEC at the concentrations evaluated.

Impact of Temperature on Sanitizer Action

As shown in Fig. 1, a higher concentration of PAA and chlorine at 32°C resulted in the highest STEC inactivation (6.23 and 6.27 log CFU/mL, respectively), whereas lower concentration of chlorine at 12°C caused the maximum STEC recovery (2.14 log CFU/mL).

At 32°C, higher sanitizer concentrations led to maximum log reduction and no significant difference was observed between the two chemistries. For the lower sanitizer concentrations at 32°C, no significant difference was observed between PAA and chlorine ($p>0.05$), but these values were significantly different from the higher concentrations ($p<0.05$). At 12°C, chlorine and PAA at higher concentrations caused the maximum STEC reduction (6.08 and 6.01 log CFU/mL respectively) with no significant difference between them ($p>0.05$). Chlorine at the lower concentrations caused the least STEC reduction (5.11 log CFU/mL). Significant difference was observed between reduction by PAA at higher and lower concentrations ($p< 0.0001$) where PAA at higher concentration led to STEC recovery of 1.23 log CFU/ mL and PAA at lower concentration resulted in 1.81 log CFU/mL. Between the lower sanitizer concentrations at 12°C, PAA resulted in a significantly higher STEC reduction when compared to chlorine ($p<0.0001$). Overall, significantly higher log reduction was observed at 32°C for all the sanitizer treatment combinations when compared to log reduction of STEC at 12°C.

Impact of Contact Time on Sanitizer Action

Highest log reduction for STEC was obtained from higher levels of chlorine and PAA concentration at both 5 and 10 min of contact time with no significant difference observed between them ($p>0.05$, Fig. 2). Lower chlorine concentration at 5 min of contact time caused the maximum survival of STEC (2.05 Log CFU/mL; $p<0.0001$) amongst any treatment combinations evaluated.

At 5 minutes of contact time, both the sanitizers at higher concentrations led to a greater STEC reduction in comparison to their lower concentrations ($p<0.0001$). Between the lower concentration of sanitizers, STEC recovery was significantly higher in the presence of chlorine ($p<0.0001$). At 10 min contact time, higher levels of sanitizers resulted in a greater STEC

reduction with no significant difference between them. STEC reduction after using chlorine and PAA at lower concentrations did not vary significantly from each other ($p>0.05$). When comparing with the lower sanitizer concentration counterparts, exposing the sanitizers for 5 min resulted in less inactivation compared to 10 min ($p<0.0001$).

Salmonella

To analyze the inactivation of *Salmonella* in agriculture water, a least square method was used where sanitizer, contact time and temperature were considered as fixed factors. Agriculture water source did not have a significant effect on the overall model ($p>0.05$) because of which it was treated as a random effect. Out of all the factors and two-way interactions amongst the fixed effects, sanitizer treatment had the most influence and contact time had the least influence on the inactivation of *Salmonella*. For the interactions analyzed, all the sanitizer treatment combinations resulted in at least 5 log reduction when compared to their respective controls (Fig. 3. and Fig. 4).

Sanitizer Effectiveness

Out of the four sanitizer treatment combinations, the most *Salmonella* inactivation was observed after using PAA and chlorine at high concentration levels (10 ppm). There was no significant difference ($p>0.05$) observed between the two sanitizers when used at this concentration. When comparing the lower concentrations of PAA (6ppm) and chlorine (4 ppm), a significant difference ($p< 0.0001$) was observed and use of PAA resulted in a lower log recovery of *Salmonella* than that observed from using chlorine (1.33 and 1.54 log CFU/mL, respectively) .

Impact of Temperature on Sanitizer Action

As shown in Fig. 3, higher levels of PAA and chlorine at both 32°C and 12°C and lower concentrations of PAA and chlorine at 32°C resulted in maximum log reduction of *S. enterica* with no significant difference amongst them ($p>0.05$). At 32°C, the activity of both the sanitizers at high and low concentrations showed similar trends and no significant difference was observed between them ($p>0.05$). Overall, a 6.03- 6.14 log CFU/mL reduction was observed amongst these treatments. At 12°C, a significant difference was observed between the higher and the lower sanitizer concentrations where higher sanitizer concentrations caused a larger log reduction of *Salmonella* as compared to their lower concentration counterparts ($p<0.0001$). At 12°C, a log reduction of 6.00- 6.06 log CFU/mL was observed in chlorine and PAA at the higher concentration. At the lower concentration, a significant difference was observed between chlorine and PAA at 12°C, resulting in 5.50 and 5.76 log CFU/mL reduction, respectively ($p<0.0001$).

Impact of Contact Time on Sanitizer Action

Overall, chlorine at higher concentration exposed for a contact time of 10 min resulted in the most inactivation, 6.27 log CFU/mL, and the lower concentration of chlorine with a contact time of 5 min led to the least log reduction of *Salmonella*,5.49 log CFU/mL (Fig. 4). At 5 min contact time, exposure of PAA and chlorine at higher concentrations and PAA at lower concentration caused the maximum *Salmonella* inactivation, 5.98, 5.92 and 5.94 log CFU/mL, respectively. No significant difference was observed between PAA at higher and lower concentrations for 5- and 10-min contact time ($p>0.05$). Exposing chlorine at higher concentration resulted in a significantly higher *Salmonella* log reduction as compared to chlorine exposed at lower concentration at both contact times ($p<0.0001$). At 10 min contact time, chlorine at higher concentration resulted in the maximum log reduction (6.27 log CFU/mL)

and was significantly higher than the rest of the treatment combinations ($p<0.0001$). When comparing both the contact times, concentration of chlorine at 10 min contact time caused significantly higher *Salmonella* reduction than chlorine at 5 min contact time for both higher and lower concentrations (Fig. 4). No significant difference ($p>0.05$) was observed in the log reduction by either concentration of PAA at both 5 and 10 minutes of contact time.

Physicochemical parameters of agricultural water sources

Table 3. mentions the physicochemical parameters of both agricultural water sources. pH levels for water sources one and two was reported as 7.06±1.61 and 7.47±0.04, respectively. For the COD levels, it was observed that source one had a much higher COD level when compared to that source two (228.5±138.5 and 68.5±0.5, respectively).

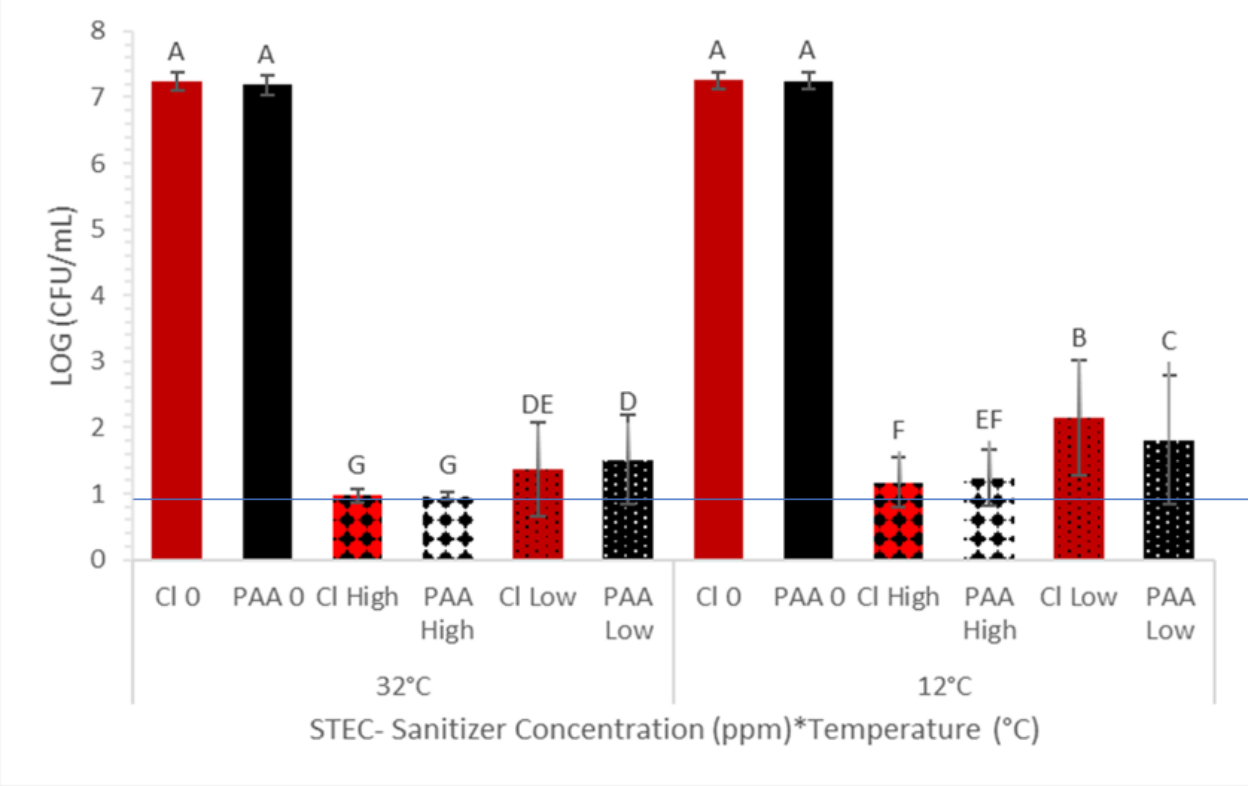

Figure 1. STEC recovered (log CFU/mL) after exposure to different sanitizer concentrations at different temperatures (32°C and 12°C). Means with different letters are significantly different ($p<0.0001$; limit of detection= 0.9 log CFU/mL). Control (Chlorine) (■), Control (PAA) (■), Chlorine at high concentration(■), PAA at high concentration (■), Chlorine at low concentration (■), PAA at low concentration (■). Limit of detection= 0.9 log CFU/mL (▬).

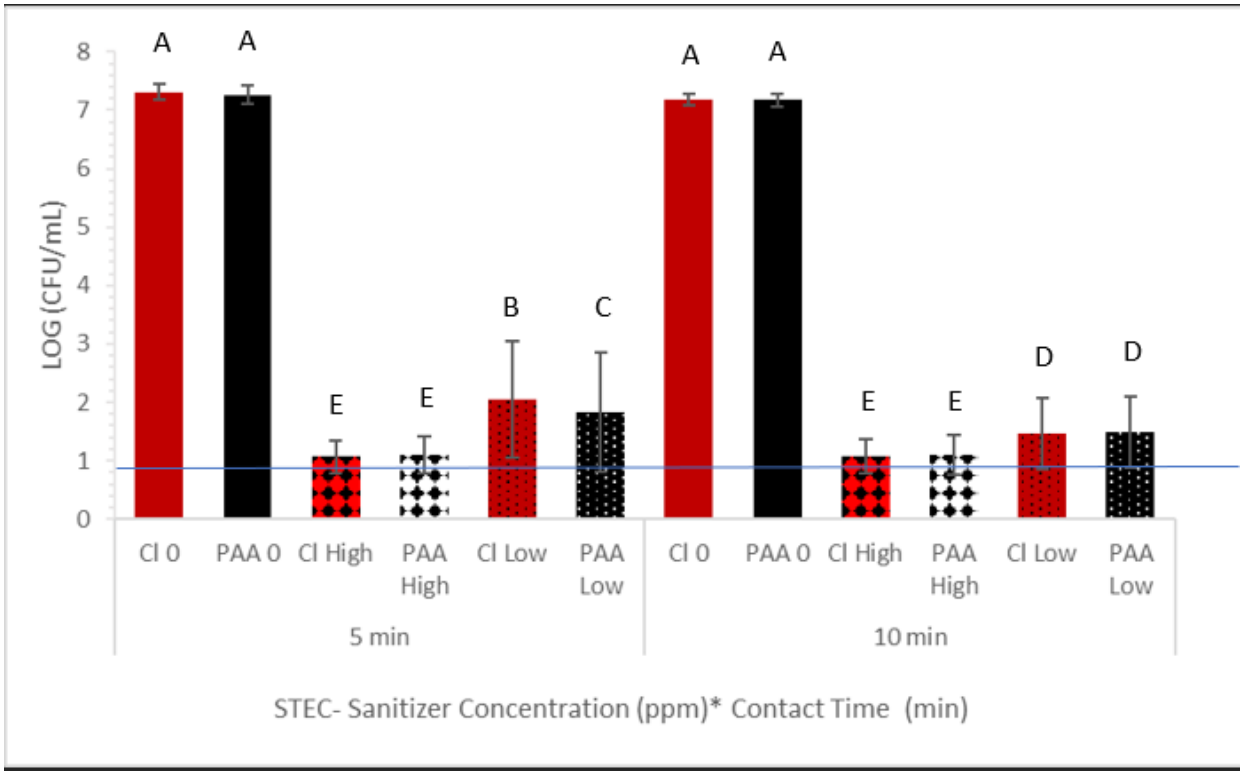

Figure 2. STEC recovered (log CFU/mL) after exposure to different sanitizer concentrations at different contact time (5min and 10min). Means with different letters are significantly different ($p<0.0001$; limit of detection= 0.9 log CFU/mL). Control (Chlorine) (■), Control (PAA) (■), Chlorine at high concentration(■), PAA at high concentration (■), Chlorine at low concentration (■), PAA at low concentration (■). Limit of detection= 0.9 log CFU/mL (■).

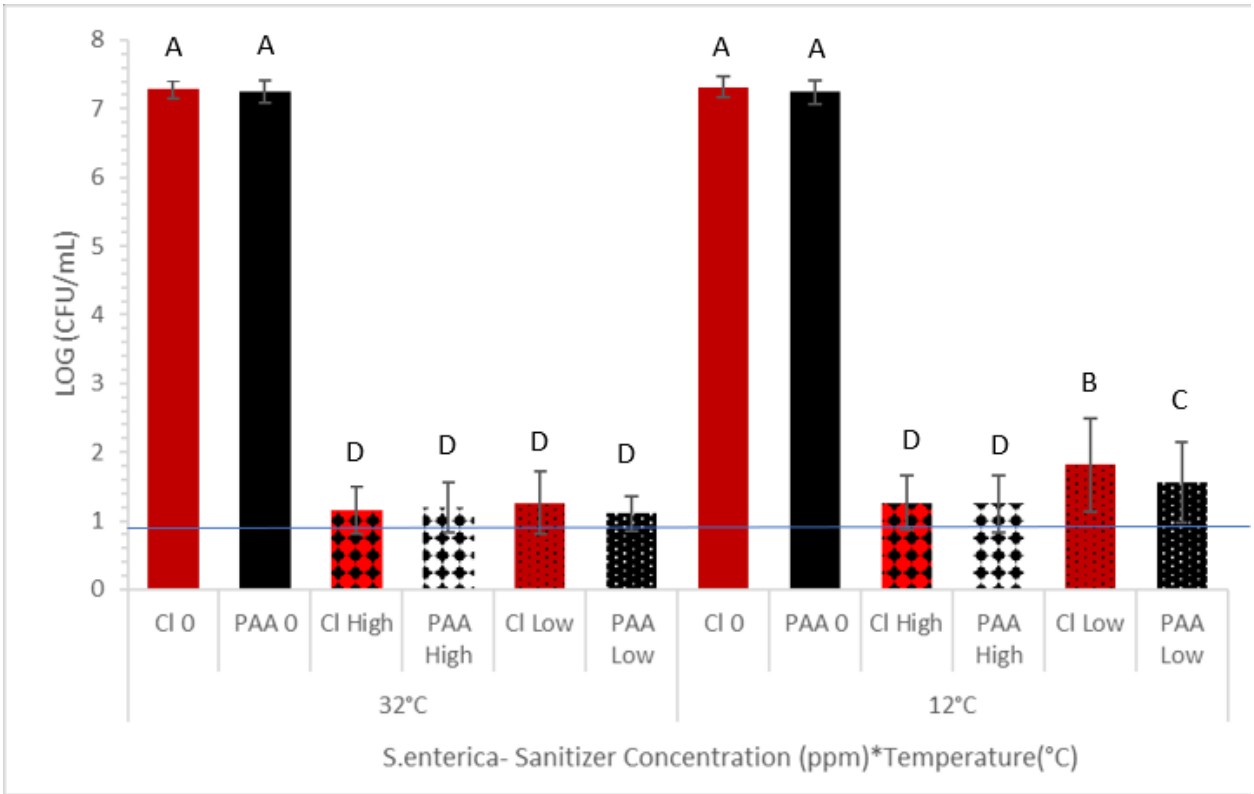

Figure 3. *Salmonella enterica* recovered (log CFU/mL) after exposure to different sanitizer concentrations at different temperatures (32°C and 12°C). Means with different letters are significantly different ($p<0.0001$; limit of detection= 0.9 log CFU/mL). Control (Chlorine) (■), Control (PAA) (■), Chlorine at high concentration(■), PAA at high concentration (■), Chlorine at low concentration (■), PAA at low concentration (■). Limit of detection= 0.9 log CFU/mL ( ).

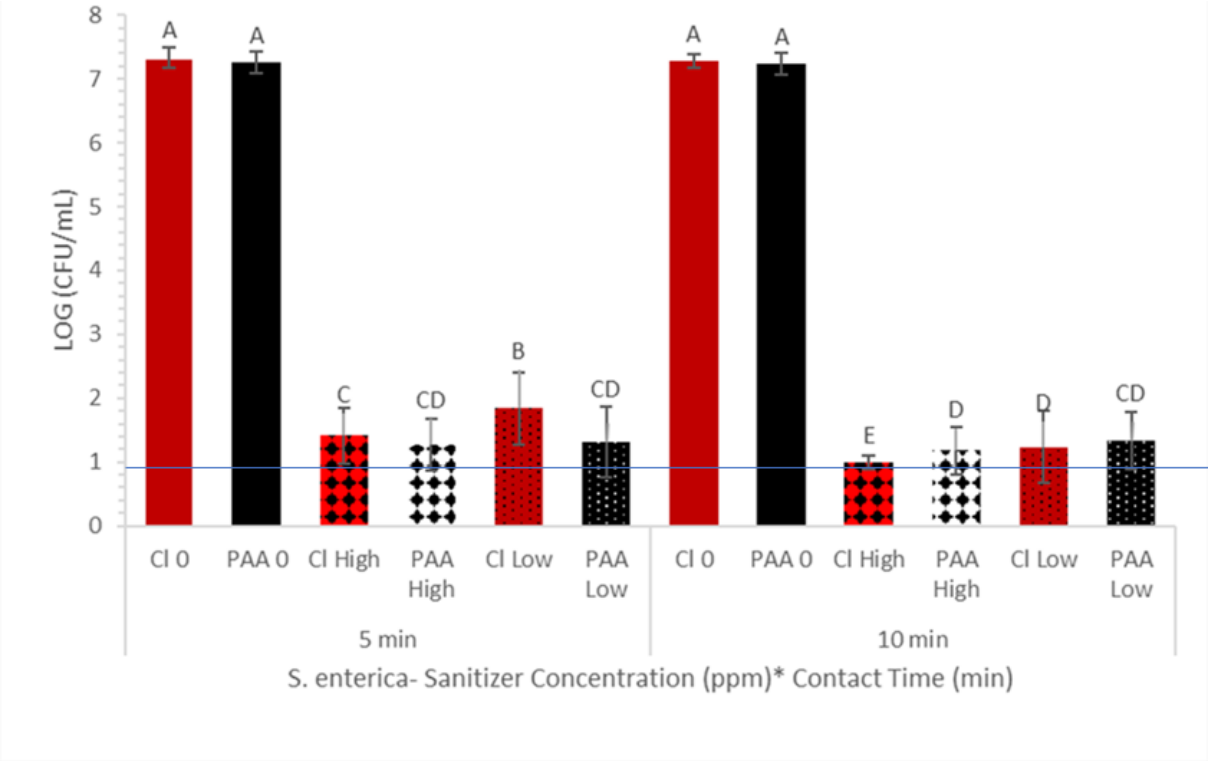

Figure 4. *Salmonella enterica* recovered (log CFU/mL) after exposure to different sanitizer concentrations at different contact time (5min and 10min). Means with different letters are significantly different ($p<0.0001$; limit of detection= 0.9 log CFU/mL). Control (Chlorine) (■), Control (PAA) (■), Chlorine at high concentration(■), PAA at high concentration (■), Chlorine at low concentration (■), PAA at low concentration (■). Limit of detection= 0.9 log CFU/mL (■).

Water Source	pH[1]	COD (ppm)	Conductivity (µS/cm)	Turbidity (FAU)	ORP (Mv)	Temperature (°C)
1	7.06±1.61	228.5±138.5	76.8±7.5	45.5±1.5	489.45±47.65	16.2±1.5
2	7.47±0.04	68.5±0.5	138.8±11.8	29.5±0.5	363.35±47.65	18.5±0.7

Table 3. Physicochemical parameters measured for two different agricultural water sources in Georgia.

[1] means with standard deviations as determined in two water samples per source.

CHAPTER 5

DISCUSSION

Sanitizer Effectiveness

When comparing the effectiveness of the sanitizer treatments applied for inactivating *Salmonella*, chlorine, and PAA at higher concentrations (10 ppm) showed the highest efficiency with no significant difference between them ($p>0.05$). However, at the lower concentrations of both the sanitizers (PAA and chlorine at 6 ppm and 4 ppm respectively), the application of PAA resulted in a significantly lower log recovery of *Salmonella* than chlorine (1.33 and 1.54 log CFU/mL respectively). A study conducted by (Krishnan et al., 2023) showed similar trends where PAA was more effective when compared to chlorine at lower concentrations (3 and 5 ppm, respectively) and at higher concentrations (7 ppm), no significant difference was observed between both the sanitizers. Analyzing the results for STEC in our study, both PAA and chlorine at higher concentrations resulted in the maximum log reduction with no significant difference between them ($p>0.05$). However, at the lower concentrations too, no significant difference was observed between the sanitizers ($p>0.05$).

Previous research has also shown the influence of pH on the efficacy of sanitizer compounds used for bacterial inactivation (Fukuzaki, 2006; Ghostlaw et al., 2020; Kishimoto et al., 2018; Kumar & Margerum, 1987; Kunigk & Almeida, 2001; Mishra et al., 2018a). For chlorine-based sanitizers, the pH of agricultural water should be near the pKa of hypochlorous acid (HOCl) i.e., 7.44 (Kishimoto et al., 2018). When chlorine reacts with water, HOCl is

released which acts as an antimicrobial (Suslow, 2021). Holding the pH of water between 6.5 and 7.5 increases the release of HOCl (Mishra et al., 2018b). At pH below 6, chlorine gas (Cl_2) is emitted whereas at higher pH (above 8), HOCl production decreases and hypochlorite ion (OCl-) release increases which further decreases the sanitizer efficiency (Fukuzaki, 2006; Kunigk & Almeida, 2001). PAA on the other hand, is efficient at a pH range of 3-7.5 and decomposes to acetic acid and oxygen which are harmless (Gehr et al., 2003; Kunigk & Almeida, 2001). At a pH lower than the pKa of PAA, it has been shown to increase the sanitizer lethality to *E. coli* O157:H7 and its stability (Ghostlaw et al., 2020). Observing Table 3, the pH for both the agricultural water sources used in this study was between the ideal range for both the sanitizers to dissociate effectively, 7.06±6.1 and 7.47±0.04 for water sources 1 and 2, respectively.

One of the major disadvantages of using chlorine over PAA is that when the organic matter present in the water is in high concentration, chlorine reacts with it and releases inefficient by-products which further increases the chlorine demand (Bonetta et al., 2021; Hassenberg et al., 2017). COD is expressed in mg oxygen consumed/L of solution and is typically used as a testing technique to evaluate overall water quality (Hu, Z. et al., 2005). By measuring the quantity of oxygen needed to convert all organic matter to carbon dioxide, COD indirectly determines the amount of oxidizable organic matter present in the water source (Kosseva, 2013). The possibility that the sanitizer may interact with organic matter rather than the desired micrograms of concern increases with a higher COD level, which implies a higher load of organic matter in the system (Van Haute et al., 2013). PAA works on oxidizing method and has a higher oxidation potential than chlorine (Zhang et al., 2019). It causes a loss of electrons from the bacterial cell wall which breaks the interaction between enzyme and protein causing rupturing of the bacterial cell (Dery et al., 2021).

Impact of Temperature on Sanitizer Action

Further, two-way interactions were analyzed between the sanitizer and temperature, which significantly affected both microorganisms. After applying the sanitizer treatments to the agricultural water inoculated with *Salmonella* at a higher temperature (32°C), PAA and chlorine at both higher and lower concentrations resulted in a maximum log reduction with no significant difference between them (Fig. 3). This shows that for inactivation of *Salmonella* at warmer temperatures, lower concentrations of chlorine and PAA (4 and 6 ppm, respectively) can be used in order to be cost-effective and reduce toxicity. However, for STEC at 32°C, PAA and chlorine at higher concentrations resulted in maximum reduction with no significant difference between them whereas, the least log reduction was obtained by applying PAA and chlorine at lower concentrations ($p>0.05$; Fig. 1).

Although, at warmer temperatures, higher sanitizer concentrations resulted in maximum STEC reduction, lower concentrations can also be applied as they also resulted in at least 5 log reduction. This can help avoid the dissociation of toxic by-products as chlorine tends to immediately react with other compounds in water (*FSIS Environmental Safety and Health Group*, June 7, 2023). In a study conducted by Zhang et al. (2022), PAA was found to be more effective at warmer temperatures when compared to sodium hypochlorite. At 12°C, for both STEC and *Salmonella*, the least reduction occurred from applying lower concentrations of chlorine (5.11 and 5.5 log CFU/mL respectively; Fig. 1 and 3). Similar trends were observed for both STEC and *Salmonella* at 12°C where higher concentrations of chlorine and PAA showed no significant difference between them ($p>0.05$) and resulted in maximum reduction and for lower concentrations, reduction by PAA was more than that by chlorine. This shows that at colder temperatures, both chlorine and PAA can be applied at higher concentrations, and at lower

concentrations, PAA is preferred over chlorine as it demonstrates slightly greater efficacy. PAA has previously been shown to be more effective than chlorine at lower temperatures (Stampi et al., 2001). The effectiveness of sanitizers increases with the increase in temperature as the reaction rate increases (Baldry, 1983; George Weber & Colonel Max Levine, 1944).

As calcium hypochlorite is used in the tablet form, the granules are fully dissolved if the water is warm enough whereas at colder temperatures it might lead to phytotoxicity (Mishra et al., 2018b). In a study by (Hassenberg et al., 2017), at a lower temperature of 2°C, *E.coli* reduction was shown to be less and took longer time when compared to its reduction at a comparatively higher temperature (15°C) after applying chlorine dioxide (ClO_2). Microbial activity of PAA was also shown by Lee and Huang (2019) to be higher at 43 °C - 46 °C when compared to the ambient temperature (Lee & Huang, 2019).

Impact of Contact Time on Sanitizer Action

Interaction of sanitizer treatments with contact time also significantly impacted the inactivation of STEC and *Salmonella*. When the sanitizer treatments were exposed for 5 min, the higher concentration of PAA and chlorine along with PAA at lower concentration resulted in a maximum log reduction of *Salmonella* with no significant difference between these sanitizer treatments (Fig. 4). A lower concentration of chlorine led to the least amount of *Salmonella* reduction when exposed for a contact time of 5 min. On the other hand, for STEC, the same trend was followed at 5 min contact time for higher concentrations where both PAA and chlorine caused maximum reduction without any significant difference, and for lower concentrations, PAA caused significantly higher reduction than chlorine (Fig. 2). When the sanitizer treatments were exposed for a contact time of 10 min, maximum log reduction for STEC was achieved by a higher concentration of chlorine and PAA with no significant difference. However, for

Salmonella, chlorine at higher concentrations showed maximum log reduction whereas PAA at higher concentrations showed a similar trend to the sanitizers at lower concentrations with no significant difference.

Overall, exposing STEC and Salmonella to the sanitizers (higher concentrations of chlorine and PAA for STEC and chlorine for *Salmonella*) for a contact time of 10 min resulted in better bacterial inactivation compared to the contact time of 5 min. In a study conducted by (Hamilton et al., 2023), where *Salmonella* and STEC reduction was analyzed after a contact time of 1, 5, and 10 min, PAA at 1 min contact time resulted in the least amount of *Salmonella* reduction whereas 54.2% (26/48) and 95.8% (46/48) of PAA treatment combinations reduced CFU/100 mL by 3 log at 5 min and 10 min, respectively. Chlorine showed a 3.35 log CFU/mL higher reduction of *Salmonella* when compared to PAA (Hamilton et al., 2023). Previous studies have shown that higher contact time reduces foodborne pathogens and indicator organisms (Ghostlaw et al., 2020; Hamilton et al., 2023; Hassenberg et al., 2017). As the contact time increases, a longer sanitizer exposure is given to the bacteria as a result of which the antimicrobial compounds dissociated from the sanitizers get a longer time to act.

Conclusion

This research demonstrates the efficacy of chlorine and PAA at different concentrations under varying temperatures and contact times to inactivate STEC and *Salmonella*. This can help the growers identify and adopt science-based water treatment strategies to reduce microbial contamination from preharvest agricultural water. There has been limited work done with the preharvest water treatment as of yet. This study is limited to evaluating only two agricultural water sources. Further research is needed to expand the number and types of surface water evaluated over several production seasons. Additional research is required that will evaluate the

toxic by-products released (if any), conducts cost comparisons for different sanitizers used at a comparable concentration, and study of the effects of interactions between different factors (pH, temperature, sanitizer concentration, contact time, COD, turbidity, ORP). Lastly, models encompassing a wide range of water quality types should be developed which provide conservative treatment efficacy predictions for *Salmonella* and STEC.

REFERENCES

Akinde, S. B., Sunday, A. A., Adeyemi, F. M., Fakayode, I. B., Oluwajide, O. O., Adebunmi, A. A., Oloke, J. K., & Adebooye, C. O. (2016). Microbes in irrigation water and fresh vegetables: Potential pathogenic bacteria assessment and implications for food safety. Applied Biosafety, 21(2), 89–97. https://doi.org/10.1177/1535676016652231/ASSET/IMAGES/LARGE/10.1177_1535676016652231-TABLE4.JPEG

Alegbeleye, O. O., Singleton, I., & Sant'Ana, A. S. (2018). Sources and contamination routes of microbial pathogens to fresh produce during field cultivation: A review. Food Microbiology, 73, 177–208. https://doi.org/10.1016/J.FM.2018.01.003

Allende, A., & Monaghan, J. (2015). Irrigation water quality for leafy crops: A perspective of risks and potential solutions. International Journal of Environmental Research and Public Health, 12(7), 7457. https://doi.org/10.3390/IJERPH120707457

Alum, Akanele, E., Urom, Chukwu, S., Ben, & Ahudie, C. M. (2016). Microbiological contamination of food: The mechanisms, impacts and prevention. International Journal of Scientific & Technology Research.

Andino, A., & Hanning, I. (2015). *Salmonella enterica*: Survival, colonization, and virulence differences among serovars. The Scientific World Journal, 2015. https://doi.org/10.1155/2015/520179

Arthur, T. M., Brichta-Harhay, D. M., Bosilevac, J. M., Kalchayanand, N., Shackelford, S. D., Wheeler, T. L., & Koohmaraie, M. (2010). Super shedding of *Escherichia coli* O157:H7 by cattle and the impact on beef carcass contamination. Meat Science, 86(1), 32–37. https://doi.org/10.1016/J.MEATSCI.2010.04.019

Baker, C. A., Rubinelli, P. M., Park, S. H., Carbonero, F., & Ricke, S. C. (2016). Shiga toxin-producing *Escherichia coli* in food: Incidence, ecology, and detection strategies. Food Control, 59, 407–419. https://doi.org/10.1016/J.FOODCONT.2015.06.011

Baldry, M. G. C. (1983). The bactericidal, fungicidal and sporicidal properties of hydrogen peroxide and peracetic acid. Journal of Applied Bacteriology, 54(3), 417–423. https://doi.org/10.1111/J.1365-2672.1983.TB02637.X

Banach, J. L., & Van Der Fels-Klerx, H. J. (2020). General interest microbiological reduction strategies of irrigation water for fresh produce. Journal of Food Protection, 83(6), 1072-1087. https://doi.org/10.4315/JFP-19-466

Barton Behravesh, C., Mody, R. K., Jungk, J., Gaul, L., Redd, J. T., Chen, S., Cosgrove, S., Hedican, E., Sweat, D., Chávez-Hauser, L., Snow, S. L., Hanson, H., Nguyen, T.-A., Sodha, S. V, Boore, A. L., Russo, E., Mikoleit, M., Theobald, L., Gerner-Smidt P, Hoekstra RM, Angulo FJ, Swerdlow DL, Tauxe RV, Griffin PM, Williams IT; Salmonella Saintpaul outbreak investigation team. (2011). 2008 outbreak of Salmonella Saintpaul infections associated with raw produce. The New England Journal of Medicine, 364(10), 918–927. https://doi.org/10.1056/NEJMoa1005741

Baudart, J., Lemarchand, K., Brisabois, A., & Lebaron, P. (2000). Diversity of *Salmonella* strains isolated from the aquatic environment as determined by serotyping and amplification of the ribosomal DNA spacer regions. Applied and Environmental Microbiology, 66(4), 1544–1552. https://doi.org/10.1128/AEM.66.4.1544-1552.2000

Berger, C. N., Sodha, S. V., Shaw, R. K., Griffin, P. M., Pink, D., Hand, P., & Frankel, G. (2010). Fresh fruit and vegetables as vehicles for the transmission of human pathogens. Environmental Microbiology, 12(9), 2385–2397. https://doi.org/10.1111/J.1462-2920.2010.02297.X

Bester, C., & Lamprecht, C. (2015). Investigating the efficacy of sodium hypochlorite, calcium hypochlorite and peracetic acid on environmental *Escherichia coli* strains. https://scholar.sun.ac.za:443/handle/10019.1/98057

Bonetta, S., Pignata, C., Bonetta, S., Amagliani, G., Brandi, G., Gilli, G., & Carraro, E. (2021). Comparison of UV, peracetic acid and sodium hypochlorite treatment in the disinfection of urban wastewater. Pathogens 2021, Vol. 10, Page 182, 10(2), 182. https://doi.org/10.3390/PATHOGENS10020182

Bowen, A., Fry, A., Richards, G., & Beauchat, L. (2006). Infections associated with cantaloupe consumption: a public health concern. Epidemiology and Infection, 134(4), 675. https://doi.org/10.1017/S0950268805005480

Brooks, J. T., Sowers, E. G., Wells, J. G., Greene, K. D., Griffin, P. M., Hoekstra, R. M., & Strockbine, N. A. (2005). Non-O157 Shiga toxin-producing *Escherichia coli* infections in the United States, 1983-2002. Journal of Infectious Diseases, 192(8), 1422–1429. https://doi.org/10.1086/466536/2/192-8-1422-TAB003.GIF

Callejón, R. M., Rodríguez-Naranjo, M. I., Ubeda, C., Hornedo-Ortega, R., Garcia-Parrilla, M. C., & Troncoso, A. M. (2015). Reported foodborne outbreaks due to fresh produce in the United States and European Union: Trends and causes. Foodborne Pathogens and Disease, 12(1), 32–38. https://doi.org/10.1089/FPD.2014.1821/ASSET/IMAGES/LARGE/FIGURE1.JPEG

Campbell, J. V., Mohle-Boetani, J., Reporter, R., Abbott, S., Farrar, J., Brandl, M., Mandrell, R., & Werner, S. B. (2001). An outbreak of *Salmonella* serotype Thompson associated with fresh cilantro. The Journal of Infectious Diseases, 183(6), 984–987. https://doi.org/10.1086/319254

Carrascosa, C., Raheem, D., Ramos, F., Saraiva, A., & Raposo, A. (2021). Microbial biofilms in the food industry—A comprehensive review. International Journal of Environmental Research and Public Health 2021, Vol. 18, Page 2014, 18(4), 2014. https://doi.org/10.3390/IJERPH18042014

Carstens, C. K., Salazar, J. K., & Darkoh, C. (2019). Multistate outbreaks of foodborne illness in the United States associated with fresh produce from 2010 to 2017. Frontiers in Microbiology, 10, 2667. https://doi.org/10.3389/FMICB.2019.02667/BIBTEX

Ceuppens, S., Johannessen, G. S., Allende, A., Tondo, E. C., El-Tahan, F., Sampers, I., Jacxsens, L., & Uyttendaele, M. (2015). Risk factors for *Salmonella*, Shiga Toxin-Producing *Escherichia coli* and *Campylobacter* occurrence in primary production of leafy greens and strawberries. International Journal of Environmental Research and Public Health 2015, Vol. 12, Pages 9809-9831, 12(8), 9809–9831. https://doi.org/10.3390/IJERPH120809809

Chang, C. Y., Hsieh, Y. H., Hsu, S. S., Hu, P. Y., & Wang, K. H. (2000). The formation of disinfection by-products in water treated with chlorine dioxide. Journal of Hazardous Materials, 79(1–2), 89–102. https://doi.org/10.1016/S0304-3894(00)00184-9

Collivignarelli, M. C., Abbà, A., Benigna, I., Sorlini, S., & Torretta, V. (2018). *Overview of the main disinfection processes for wastewater and drinking water treatment plants. Sustainability (Switzerland), 10(1). https://doi.org/10.3390/SU10010086*
Crum-Cianfl One, N. F. (2008). *Salmonellosis* and the Gastrointestinal Tract: More Than Just Peanut Butter.

da Conceição, R. de C. dos S., Sturbelle, R. T., Timm, C. D., & Leite, F. P. L. (2015). Inducers and autoinducers on *Salmonella enterica* serovar Typhimurium motility, growth and gene expression. Ciencia Rural, 45(12), 2201–2206. https://doi.org/10.1590/0103-8478CR20150295

Declan Iwu, C., & Okoh, A. I. (2019). Preharvest transmission routes of fresh produce associated bacterial pathogens with outbreak potentials: A review. https://doi.org/10.3390/ijerph16224407

Dery, J. L., Choppakatla, V., Sughroue, J., & Rock, C. (2021). Minimizing risks: Use of surface water in pre-harvest agricultural irrigation; Part III: Peroxyacetic acid (PAA) treatment methods. https://extension.arizona.edu/sites/extension.arizona.edu/files/pubs/az1884-2021.pdf (last accessed 7.15.23).

Dirk Van Elsas, J., Semenov, A. V, Costa, R., & Trevors, J. T. (2011a). Mini review survival of *Escherichia coli* in the environment: Fundamental and public health aspects. The ISME Journal, 5, 173–183. https://doi.org/10.1038/ismej.2010.80

Ehuwa, O., Jaiswal, A. K., & Jaiswal, S. (2021). *Salmonella*, food safety and food handling practices. Foods, 10(5). https://doi.org/10.3390/FOODS10050907

Electronic public release version module 5: Agricultural water. (2019).

Erickson, M. C., & Doyle, M. P. (2007). Food as a vehicle for transmission of Shiga toxin-producing *Escherichia coli*. Journal of Food Protection, 70(10), 2426–2449. https://doi.org/10.4315/0362-028X-70.10.2426

Fàbrega, A., & Vila, J. (2013). *Salmonella enterica* serovar Typhimurium skills to succeed in the host: Virulence and regulation. Clinical Microbiology Reviews, 26(2), 308–341. https://doi.org/10.1128/CMR.00066-12

Faour-Klingbeil, D., Murtada, M., Kuri, V., & Todd, E. C. D. (2016). Understanding the routes of contamination of ready-to-eat vegetables in the Middle East. Food Control, 62, 125–133. https://doi.org/10.1016/J.FOODCONT.2015.10.024

Farrokh, C., et al., Review of Shiga-toxin-producing Escherichia coli (STEC) and their significance in dairy production. International journal of food microbiology, 2013. 162(2): p. 190-212. https://doi.org/10.1016/j.ijfoodmicro.2012.08.008

Fatica, M. K., & Schneider, K. R. (2011). *Salmonella* and produce: Survival in the plant environment and implications in food safety. Http://Dx.Doi.Org/10.4161/Viru.2.6.17880, 2(6), 573–579. https://doi.org/10.4161/VIRU.2.6.17880

Ferrari, R. G., Rosario, D. K. A., Cunha-Neto, A., Mano, S. B., Figueiredo, E. E. S., & Conte-Juniora, C. A. (2019). Worldwide epidemiology of *Salmonella* serovars in animal-based foods: A meta-analysis. Applied and Environmental Microbiology, 85(14). https://doi.org/10.1128/AEM.00591-19

Ford, L., Moffatt, C. R. M., Fearnley, E., Miller, M., Gregory, J., Sloan-Gardner, T. S., Polkinghorne, B. G., Bell, R., Franklin, N., Williamson, D. A., Glass, K., & Kirk, M. D. (2018). The Epidemiology of *Salmonella enterica* outbreaks in Australia, 2001–2016. Frontiers in Sustainable Food Systems, 2, 86. https://doi.org/10.3389/FSUFS.2018.00086/BIBTEX

Fukuzaki, S., Mechanisms of actions of sodium hypochlorite in cleaning and disinfection processes. Biocontrol science, 2006. 11(4): p. 147-157.

García, A. V, Hirt, H., Kovalchuk, I., & Denby, K. (2014). *Salmonella enterica* induces and subverts the plant immune system. https://doi.org/10.3389/fmicb.2014.00141

Gehr, R., Wagner, M., Veerasubramanian, P., & Payment, P. (2003). Disinfection efficiency of peracetic acid, UV and ozone after enhanced primary treatment of municipal wastewater. Water Research, 37(19), 4573–4586. https://doi.org/10.1016/S0043-1354(03)00394-4

George Weber, C. R., & Colonel Max Levine, L. (1944). Factors Affecting germicidal efficiency of chlorine and chloramine. Am J Public Health Nations Health., 34.

Ghostlaw, T., Corradini, M. G., Autio, W. R., & Kinchla, A. J. (2020). Impact of various postharvest wash water conditions on the performance of peracetic acid against *Escherichia coli* O157:H7 over time. Food Control, 109, 106891. https://doi.org/10.1016/J.FOODCONT.2019.106891

Goodson, M., & Rowbury, R. J. (1989). Habituation to normally lethal acidity by prior growth of *Escherichia coli* at a sub-lethal acid pH value. Letters in Applied Microbiology, 8(2), 77–79. https://doi.org/10.1111/J.1472-765X.1989.TB00227.X

Gray, N. F. (2014). Free and combined chlorine. Microbiology of Waterborne Diseases: Microbiological Aspects and Risks: Second Edition, 571–590. https://doi.org/10.1016/B978-0-12-415846-7.00031-7

Greene, S. K., Daly, E. R., Talbot, E. A., Demma, L. J., Holzbauer, S., Patel, N. J., Hill, T. A., Walderhaug, M. O., Hoekstra, R. M., Lynch, M. F., & Painter, J. A. (2008). Recurrent multistate outbreak of *Salmonella* Newport associated with tomatoes from contaminated fields, 2005. Epidemiology and Infection, 136(2), 157–165. https://doi.org/10.1017/S095026880700859X

Griffin, P. M., & Tauxe, R. V. (1991). The epidemiology of infections caused by *Escherichia coli* O157:H7, other enterohemorrhagic *E. coli*, and the associated hemolytic uremic syndrome. Epidemiologic Reviews, 13(1), 60–98. https://doi.org/10.1093/OXFORDJOURNALS.EPIREV.A036079

Gurtler, J. B., & Gibson, K. E. (2022). Irrigation water and contamination of fresh produce with bacterial foodborne pathogens. Current Opinion in Food Science, 47, 100889. https://doi.org/10.1016/J.COFS.2022.100889

Gyles, C. L. (2007). Shiga toxin-producing *Escherichia coli*: An overview. Journal of Animal Science, 85(suppl_13), E45–E62. https://doi.org/10.2527/JAS.2006-508

Haley, B. J., Cole, D. J., & Lipp, E. K. (2009). Distribution, diversity, and seasonality of waterborne *Salmonellae* in a rural watershed. Applied and Environmental Microbiology, 75(5), 1248. https://doi.org/10.1128/AEM.01648-08

Murphy, C.M., et al., Sanitizer Type and Contact Time Influence Salmonella Reductions in Preharvest Agricultural Water Used on Virginia Farms. Journal of Food Protection, 2023: p. 100110.https://doi.org/10.1016/j.jfp.2023.100110

Hanning, I. B., Nutt, J. D., & Ricke, S. C. (2009). Salmonellosis outbreaks in the United States due to fresh produce: Sources and potential intervention measures. Foodborne Pathogens and Disease, 6(6), 635–648. https://doi.org/10.1089/FPD.2008.0232

Hassenberg, K., Geyer, M., Mauerer, M., Praeger, U., & Herppich, W. B. (2017). Influence of temperature and organic matter load on chlorine dioxide efficacy on *Escherichia coli* inactivation. LWT - Food Science and Technology, 79, 349–354. https://doi.org/10.1016/J.LWT.2017.01.030

Hautefort, I., Proença, M. J., & Hinton, J. C. D. (2003). Single-copy green fluorescent protein gene fusions allow accurate measurement of *Salmonella* gene expression in vitro and during infection of mammalian cells. Applied and Environmental Microbiology, 69(12), 7480–7491. https://doi.org/10.1128/AEM.69.12.7480-7491.2003/ASSET/54A0C3D1-3743-4EC1-A08A-AA104D8E3428/ASSETS/GRAPHIC/AM1231137006.JPEG

Havelaar, A. H., Brul, S., de Jong, A., de Jonge, R., Zwietering, M. H., & ter Kuile, B. H. (2010). Future challenges to microbial food safety. International Journal of Food Microbiology, 139(Supplement. 1). https://doi.org/10.1016/J.IJFOODMICRO.2009.10.015

Hoff, C., Higa, J., Patel, K., Gee, E., Wellman, A., Vidanes, J., Holland, A., Kozyreva, V., Zhu, J., Mattioli, M., Roundtree, A., McFadden, K., Whitlock, L., Wise, M., Gieraltowski, L., & Schwensohn, C. (2021). Notes from the field: An outbreak of *Escherichia coli* O157:H7 infections linked to romaine lettuce exposure — United States, 2019. MMWR. Morbidity and Mortality Weekly Report, 70(18), 689–690. https://doi.org/10.15585/MMWR.MM7018A3

Homas, T., Oyce, G. B., Werdlow, A. L. S., Atricia, P., & Riffin, M. G. (1995). *Escherichia coli* O157:H7 and the hemolytic–uremic syndrome. https://doi.org/10.1056/NEJM199508103330608

Hu, Z., and D. Grasso. 2005. water analysis, Chemical Oxygen Demand, p. 325–330. In P. Worsfold, A. Townshend, and C. Poole (eds.), Encyclopedia of Analytical Science (Second Edition). Elsevier, Oxford.

Hurley, D., et al., *Salmonella*–host interactions–modulation of the host innate immune system. Frontiers in immunology, 2014. 5: p. 481. https://doi.org/10.3389/fimmu.2014.00481

Ibarra, J.A., et al., Induction of *Salmonella* pathogenicity island 1 under different growth conditions can affect *Salmonella*–host cell interactions in vitro. Microbiology, 2010. 156(Pt 4): p. 1120. https://doi.org/10.1099/mic.0.032896-0

FSIS Environmental Safety and Health Group. (2023). Chlorine health hazard information sheet. https://www.fsis.usda.gov/sites/default/files/media_file/2020-08/Chlorine.pdf (last accessed 7.15.23)

Islam, M., Doyle, M. P., Phatak, S. C., Millner, P., & Jiang, X. (2004). Persistence of enterohemorrhagic *Escherichia coli* O157:H7 in soil and on leaf lettuce and parsley grown in fields treated with contaminated manure composts or irrigation water. Journal of Food Protection, 67(7), 1365–1370. https://doi.org/10.4315/0362-028X-67.7.1365

Jackson, B. R., Griffin, P. M., Cole, D., Walsh, K. A., & Chai, S. J. (2013). Outbreak-associated *Salmonella enterica* serotypes and food commodities, United States, 1998–2008. Emerging Infectious Diseases, 19(8), 1239. https://doi.org/10.3201/EID1908.121511

Jung, Y., Jang, H., & Matthews, K. R. (2014). Effect of the food production chain from farm practices to vegetable processing on outbreak incidence. Microbial Biotechnology, 7(6), 517–527. https://doi.org/10.1111/1751-7915.12178

Kaper, J. B., Nataro, J. P., & Mobley, H. L. T. (2004). Pathogenic *Escherichia coli*. Nature Reviews| *Microbiology*, *2*, 3. https://doi.org/10.1038/nrmicro818

Kintz, E., Brainard, J., Hooper, L., & Hunter, P. (2017). Transmission pathways for sporadic Shiga-toxin producing *E. coli* infections: A systematic review and meta-analysis. International Journal of Hygiene and Environmental Health, 220(1), 57–67. https://doi.org/10.1016/J.IJHEH.2016.10.011

Kishimoto, N., Katayama, Y., Kato, M., & Otsu, H. (2018). Technical feasibility of UV/electro-chlorine advanced oxidation process and pH response. Chemical Engineering Journal, 334, 2363–2372. https://doi.org/10.1016/J.CEJ.2017.11.108

Kitis, M. (2004a). Disinfection of wastewater with peracetic acid: A review. Environment International, 30(1), 47–55. https://doi.org/10.1016/S0160-4120(03)00147-8

Klontz, K. C., Klontz, J. C., Mody, R. K., & Hoekstra, R. M. (2010). Analysis of tomato and jalapeño and serrano pepper imports into the United States from Mexico before and during a national outbreak of *Salmonell*a serotype Saintpaul infections in 2008. Journal of Food Protection, 73(11), 1967–1974. https://doi.org/10.4315/0362-028X-73.11.1967

Koivunen, J., & Heinonen-Tanski, H. (2005). Inactivation of enteric microorganisms with chemical disinfectants, UV irradiation and combined chemical/UV treatments. Water Research, 39(8), 1519–1526. https://doi.org/10.1016/J.WATRES.2005.01.021

Kosseva, M. R. 2013. Chapter 3 - Sources, characterization, and composition of food industry wastes, p. 37–60. *In* M.R. Kosseva, and C. Webb (edition), Food Industry Wastes. Academic Press, San Diego.

Kozak, G., et al., Foodborne outbreaks in Canada linked to produce: 2001 through 2009. Journal of Food Protection, 2013. 76(1): p. 173-183. https://doi.org/10.4315/0362-028X.JFP-12-126

Krishnan, A., Kogan, C., Peters, R. T., Thomas, E. L., & Critzer, F. (2021). Microbial and physicochemical assessment of irrigation water treatment methods. Journal of Applied Microbiology, 131(3), 1555–1562. https://doi.org/10.1111/JAM.15043

Krishnan, A., Xu, X., Sanchez-Tamayo, M., Mishra, A., & Critzer, F. (2023). Impact of chlorine and peracetic acid on inactivation of *Salmonella*, *Escherichia coli*, and *Listeria monocytogenes* in agricultural water. Science of The Total Environment, 2023. 885: p. 163884. https://doi.org/10.2139/SSRN.4381379

Kroupitski, Y., Golberg, D., Belausov, E., Pinto, R., Swartzberg, D., Granot, D., & Sela, S. (2009). Internalization of *Salmonella enterica* in leaves is induced by light and involves chemotaxis and penetration through open stomata. Applied and Environmental Microbiology, 75(19), 6076–6086. https://doi.org/10.1128/AEM.01084-09/SUPPL_FILE/MOVIE_3_DARK.ZIP

Kumar, K., & Margerum, D. W. (1987). Kinetics and mechanism of general-acid-assisted oxidation of bromide by hypochlorite and hypochlorous acid. Inorganic Chemistry, 26(16), 2706–2711. https://doi.org/10.1021/IC00263A030/ASSET/IC00263A030.FP.PNG_V03

Kunigk, L., & Almeida, M. C. B. (2001). Action of peracetic acid on *Escherichia coli* and *Staphylococcus aureus* in suspension or settled on stainless steel surfaces. Brazilian Journal of Microbiology, 32(1), 38–41. https://doi.org/10.1590/S1517-83822001000100009

Kunigk, L., Gomes, D. R., Forte, F., Vidal, K. P., Gomes, L. F., & Sousa, P. F. (2001). The influence of temperature on the decomposition kinetics of peracetic acid in solutions. Brazilian Journal of Chemical Engineering, 18(2), 217–220. https://doi.org/10.1590/S0104-66322001000200009

Kurtz, J. R., Goggins, J. A., & McLachlan, J. B. (2017). *Salmonella* infection: Interplay between the bacteria and host immune system. Immunology Letters, 190, 42–50. https://doi.org/10.1016/J.IMLET.2017.07.006

Lapidot, A., & Yaron, S. (2009). Transfer of *Salmonella enterica* serovar Typhimurium from contaminated irrigation water to parsley is dependent on curli and cellulose, the biofilm matrix components. Journal of Food Protection, 72(3), 618–623. https://doi.org/10.4315/0362-028X-72.3.618

Lee, W. H., Jeon, Y., Chung, J. S., Shim, W. J., Shin, M. J., Han, S. G., Baek, S., Kim, J., Kim, J., Chung, P. S., Lee, W. H., Jeon, Y., Chung, J. S., Shim, W. J., Shin, M. J., Han, S. G., Baek, S., Kim, J., Kim, J., & Chung, P. S. (2021). Global genomic variations of pathogenic bacteria

Salmonella reading. Computational Molecular Bioscience, 11(3), 57–68. https://doi.org/10.4236/CMB.2021.113004

Lee, W. N., & Huang, C. H. (2019). Formation of disinfection byproducts in wash water and lettuce by washing with sodium hypochlorite and peracetic acid sanitizers. Food Chemistry: X, 1, 100003. https://doi.org/10.1016/J.FOCHX.2018.100003

León, J. S., Jaykus, L. A., & Moe, C. L. (2008). Food safety issues and the microbiology of fruits and vegetables. Microbiologically Safe Foods, 255–290. https://doi.org/10.1002/9780470439074.CH12

Leonardo Iniguez, A., Dong, Y., Carter, H. D., Ahmer, B. M. M., Stone, J. M., & Triplett, E. W. (2005). Regulation of enteric endophytic bacterial colonization by plant defenses. / 169 MPMI, 18(2), 169–178. https://doi.org/10.1094/MPMI

Levantesi, C., Bonadonna, L., Briancesco, R., Grohmann, E., Toze, S., & Tandoi, V. (2011). *Salmonella* in surface and drinking water: Occurrence and water-mediated transmission. Food Research International, 2012. 45(2): p. 587-602.https://doi.org/10.1016/j.foodres.2011.06.037

Levine, M. M. (1987). *Escherichia coli* that cause diarrhea: enterotoxigenic, enteropathogenic, enteroinvasive, enterohemorrhagic, and enteroadherent. The Journal of Infectious Diseases, 155(3), 377–381. https://doi.org/10.1093/INFDIS/155.3.377

Leyer, G. J., Wang, L. L., & Johnson, E. A. (1995). Acid adaptation of *Escherichia coli* O157:H7 increases survival in acidic foods. Applied and Environmental Microbiology, 61(10), 3752–3755. https://doi.org/10.1128/AEM.61.10.3752-3755.1995

Liang, H., Tian, J. Y., He, W. J., Han, H. D., Chen, Z. L., & Li, G. B. (2009). Combined preoxidation by permanganate and chlorine in enhancing the treatment of surface water. Journal of Chemical Technology & Biotechnology, 84(8), 1229–1233. https://doi.org/10.1002/JCTB.2163

Luukkonen, T., & Pehkonen, S. O. (2017). Peracids in water treatment: A critical review. Http://Dx.Doi.Org/10.1080/10643389.2016.1272343, 47(1), 1–39. https://doi.org/10.1080/10643389.2016.1272343

Lynch, M. F., Tauxe, R. V., & Hedberg, C. W. (2009). The growing burden of foodborne outbreaks due to contaminated fresh produce: risks and opportunities. Epidemiology and Infection, 137(3), 307–315. https://doi.org/10.1017/S0950268808001969

Ma, L., Zhang, G., & Doyle, M. P. (2011). Green fluorescent protein labeling of *Listeria*, *Salmonella*, and *Escherichia coli* O157:H7 for safety-related studies. *Plos one, 6*(4), e18083. https://doi.org/10.1371/JOURNAL.PONE.0018083

Machado-Moreira, B., Richards, K., Brennan, F., Abram, F., & Burgess, C. M. (2019). Microbial contamination of fresh produce: What, where, and how? Comprehensive Reviews in Food Science and Food Safety, 18(6), 1727–1750. https://doi.org/10.1111/1541-4337.12487

Matthews, K. R. (2013). Sources of enteric pathogen contamination of fruits and vegetables: Future directions of research. Stewart Postharvest Review, 9(1). https://doi.org/10.2212/SPR.2013.1.2

McGhie, E. J., Brawn, L. C., Hume, P. J., Humphreys, D., & Koronakis, V. (2009). *Salmonella* takes control: Effector-driven manipulation of the host. Current Opinion in Microbiology, 12(1), 117–124. https://doi.org/10.1016/J.MIB.2008.12.001

McQuiston, J. R., Fields, P. I., Tauxe, R. V., & Logsdon, J. M. (2008). Do *Salmonella* carry spare tyres? Trends in Microbiology, 16(4), 142–148. https://doi.org/10.1016/J.TIM.2008.01.009
Migliaccio, K. W., Boman, B., & Clark, G. A. (2009). Treating Irrigation Systems with Chlorine. EDIS, 2009(6). http://edis.ifas.ufl.edu.

Mishra, V., Abrol, G. S., & Dubey, N. (2018a). Sodium and calcium hypochlorite as postharvest disinfectants for fruits and vegetables. Postharvest Disinfection of Fruits and Vegetables, 253–272. https://doi.org/10.1016/B978-0-12-812698-1.00014-5

Mody, R. K., Greene, S. A., Gaul, L., Sever, A., Pichette, S., Zambrana, I., Dang, T., Gass, A., Wood, R., Herman, K., Cantwell, L. B., Falkenhorst, G., Wannemuehler, K., Hoekstra, R. M., Mccullum, I., Cone, A., Franklin, L., Austin, J., Delea, K., Behravesh C.B., Sodha S.V., Yee J.C., Emanuel B., Al-Khaldi S.F., Jefferson V., Williams I.T., Griffin P.M., Swerdlow, D. L. (2011). National outbreak of *Salmonella* serotype Saintpaul infections: Importance of Texas restaurant investigations in implicating jalapeño peppers. PLoS One, 2011. 6(2): p. e16579. https://doi.org/10.1371/journal.pone.0016579

Monarca, S., Richardso, S. D., Feretti, D., Grottolo, M., Thruston, A. D., Zani, C., Navazio, G., Ragazzo, P., Zerbini, I., & Alberti, A. (2002). Mutagenicity and disinfection by-products in surface drinking water disinfected with peracetic acid. Environmental Toxicology and Chemistry, 21(2), 309–318. https://doi.org/10.1002/ETC.5620210212

Nataro, J. P., & Kaper, J. B. (1998). Diarrheagenic *Escherichia coli*. Clinical Microbiology Reviews, 11(1), 142–201. https://doi.org/10.1128/CMR.11.1.142
National Outbreak Reporting System (NORS) Dashboard | CDC. (2018). https://wwwn.cdc.gov/norsdashboard/

Newell, D. G., & La Ragione, R. M. (2018). Enterohaemorrhagic and other Shiga toxin-producing *Escherichia coli* (STEC): Where are we now regarding diagnostics and control strategies? Transboundary and Emerging Diseases, 65, 49–71. https://doi.org/10.1111/TBED.12789

Nguyen, L., A. Graffe, and T. Candy. Peracetic acid as a method of effluent wastewater disinfection in Langley, BC. in WEFTEC 2014. 2014. Water Environment Federation.

Nizer, W. S. da C., Inkovskiy, V., & Overhage, J. (2020). Surviving reactive chlorine stress: responses of gram-negative bacteria to hypochlorous acid. Microorganisms 2020, Vol. 8, Page 1220, 8(8), 1220. https://doi.org/10.3390/MICROORGANISMS8081220

Oron, G., Armon, R., Mandelbaum, R., Manor, Y., Campos, C., Gillerman, L., Salgot, M., Gerba, C., Klein, I., & Enriquez, C. (2001). Secondary wastewater disposal for crop irrigation with minimal risks. Water Science and Technology, 43(10), 139–146. https://doi.org/10.2166/WST.2001.0603

Pachepsky, Y., Shelton, D. R., McLain, J. E. T., Patel, J., & Mandrell, R. E. (2011). Irrigation waters as a source of pathogenic microorganisms in produce: A review. Advances in Agronomy, 113, 75–141. https://doi.org/10.1016/B978-0-12-386473-4.00002-6

Podolak, R., Enache, E., Stone, W., Black, D. G., & Elliott, P. H. (2010). Sources and risk factors for contamination, survival, persistence, and heat resistance of *Salmonella* in low-moisture foods. Journal of Food Protection, 73(10), 1919–1936. https://doi.org/10.4315/0362-028X-73.10.1919

Rabsch, W., Andrews, H. L., Kingsley, R. A., Prager, R., Tschäpe, H., Adams, L. G., & Bäumler, A. J. (2002). *Salmonella enterica* serotype Typhimurium and its host-adapted variants. Infection and Immunity, 70(5), 2249–2255. https://doi.org/10.1128/IAI.70.5.2249-2255.2002

Ragazzo, P., Chiucchini, N., Piccolo, V., Spadolini, M., Carrer, S., Zanon, F., & Gehr, R. (2020). Wastewater disinfection: Long-term laboratory and full-scale studies on performic acid in comparison with peracetic acid and chlorine. Water Research, 184. https://doi.org/10.1016/J.WATRES.2020.116169

Rahman, M. T. (2015). Salmonellosis: A major foodborne disease of global significance. Beverage and food world, 42(12), 21–24. https://www.researchgate.net/publication/288827348

Rana, K., Ranjan Nayak, S., Bihary, A., Ku Sahoo, A., Charan Mohanty, K., ku Palo, S., Sahoo, D., Pati, S., & Dash, P. (2021). Association of quorum sensing and biofilm formation with S*almonella* virulence: Story beyond gathering and cross-talk. Archives of Microbiology, 203, 5887–5897. https://doi.org/10.1007/s00203-021-02594-y

Rangel, J. M., Sparling, P. H., Crowe, C., Griffin, P. M., & Swerdlow, D. L. (2005). Epidemiology of *Escherichia coli* O157:H7 outbreaks, United States, 1982-2002. Emerging Infectious Diseases, 11(4), 603–609. https://doi.org/10.3201/EID1104.040739

Rudd, T., & Hopkinson, L. M. (1989). Comparison of disinfection techniques for sewage and sewage effluents. Water and Environment Journal, 3(6), 612–618. https://doi.org/10.1111/J.1747-6593.1989.TB01443.X

Russell, A. D. (2003). Similarities and differences in the responses of microorganisms to biocides. Journal of Antimicrobial Chemotherapy, 52(5), 750–763. https://doi.org/10.1093/JAC/DKG422
Santoro, D., Gehr, R., Bartrand, T. A., Liberti, L., Notarnicola, M., Dell'Erba, A., Falsanisi, D., & Haas, C. N. (2007). Wastewater disinfection by peracetic acid: Assessment of models for tracking residual measurements and inactivation. Water Environment Research, 79(7), 775–787. https://doi.org/10.2175/106143007X156817

Scallan, E., Griffin, P. M., Angulo, F. J., Tauxe, R. V., & Hoekstra, R. M. (2011). Foodborne illness acquired in the United States--unspecified agents. Emerging Infectious Diseases, 17(1), 16–22. https://doi.org/10.3201/EID1701.091101P2

Scallan, E., Hoekstra, R. M., Angulo, F. J., Tauxe, R. V., Widdowson, M. A., Roy, S. L., Jones, J. L., & Griffin, P. M. (2011). Foodborne illness acquired in the United States—Major Pathogens - Volume 17, Number 1—January 2011 - Emerging Infectious Diseases journal - CDC. Emerging Infectious Diseases, 17(1), 7–15. https://doi.org/10.3201/EID1701.P11101

Schikora, A., Garcia, A. V, & Hirt, H. (2012). Plants as alternative hosts for *Salmonella*. Trends in Plant Science, 17(5). https://doi.org/10.1016/j.tplants.2012.03.007

Silbergleit, M., Vasquez, A. A., Miller, C. J., Sun, J., & Kato, I. (2020). Oral and intestinal bacterial exotoxins: Potential linked to carcinogenesis. Progress in Molecular Biology and Translational Science, 171, 131–193. https://doi.org/10.1016/BS.PMBTS.2020.02.004

Sisti, M., Albano, A., & Brandi, G. (1998). Bactericidal effect of chlorine on motile *Aeromonas spp.* in drinking water supplies and influence of temperature on disinfection efficacy. Letters in Applied Microbiology, 26(5), 347–351. https://doi.org/10.1046/J.1472-765X.1998.00346.X

Small, D. A., Chang, W., Toghrol, F., & Bentley, W. E. (2007). Comparative global transcription analysis of sodium hypochlorite, peracetic acid, and hydrogen peroxide on *Pseudomonas aeruginosa*. Applied Microbiology and Biotechnology, 76(5), 1093–1105. https://doi.org/10.1007/S00253-007-1072-Z/FIGURES/3

Söderström, A., Österberg, P., Lindqvist, A., Jönsson, B., Lindberg, A., Blide Ulander, S., Welinder-Olsson, C., Löfdahl, S., Kaijser, B., De Jong, B., Kühlmann-Berenzon, S., Boqvist, S., Eriksson, E., Szanto, E., Andersson, S., Allestam, G., Hedenström, I., Ledet Muller, L., &

Andersson, Y. (2008). A large *Escherichia coli* O157 outbreak in Sweden associated with locally produced lettuce. Foodborne Pathogens and Disease, 5(3), 339–349. https://doi.org/10.1089/FPD.2007.0065

Somani, S. B., Ingole, N. W., & Kulkarni, N. S. (2011). Disinfection of water by using sodium chloride (NaCl) and sodium hypochlordie (NaOCl).

Stampi, S., De Luca, G., & Zanetti, F. (2001). Evaluation of the efficiency of peracetic acid in the disinfection of sewage effluents. Journal of Applied Microbiology, 91(5), 833–838. https://doi.org/10.1046/J.1365-2672.2001.01451.X

Steele, M., & Odumeru, J. (2004). Irrigation water as source of foodborne pathogens on fruit and vegetables. Journal of Food Protection, 67(12), 2839–2849. https://doi.org/10.4315/0362-028X-67.12.2839

Sultana, M., Anwar Hossain, M., Jahangir Alam, M., Latiful Bari, M., Liu huanliliu, H., Baoguang Li, fdahhsgov, Liu, H., Whitehouse, C. A., & Li, B. (2018). Article 159 B (2018) Presence and persistence of S*almonella* in water: The impact on microbial quality of water and food safety. Front. Public Health, 6, 159. https://doi.org/10.3389/fpubh.2018.00159

Sun, P., Zhang, T., Mejia-Tickner, B., Zhang, R., Cai, M., & Huang, C. H. (2018). Rapid disinfection by peracetic acid combined with UV irradiation. Environmental Science and Technology Letters, 5(6), 400–404. https://doi.org/10.1021/ACS.ESTLETT.8B00249/SUPPL_FILE/EZ8B00249_SI_001.PDF

Suslow, T. V. (2021). Water disinfection a practical approach to calculating dose values for preharvest and postharvest applications. http://anrcatalog.ucdavis.edu (last accessed 7.14.23)

Tack, D. M., Kisselburgh, H. M., Richardson, L. C., Geissler, A., Griffin, P. M., Payne, D. C., & Gleason, B. L. (2021). Shiga Toxin-Producing *Escherichia coli* outbreaks in the United States, 2010-2017. Microorganisms, 9(7). https://doi.org/10.3390/MICROORGANISMS9071529

Tchaptchet, S., & Hansen, J. (2011). The Yin and Yang of host-commensal mutualism. *Https://Doi.Org/10.4161/Gmic.19089*, *2*(6), 347–352. https://doi.org/10.4161/GMIC.19089
Thunberg, R. L., Tran, T. T., Bennett, R. W., Matthews, R. N., & Belay, N. (2002). Microbial evaluation of selected fresh produce obtained at retail markets. Journal of Food Protection, 65(4), 677–682. https://doi.org/10.4315/0362-028X-65.4.677

Uyttendaele, M., Jaykus, L. A., Amoah, P., Chiodini, A., Cunliffe, D., Jacxsens, L., Holvoet, K., Korsten, L., Lau, M., McClure, P., Medema, G., Sampers, I., & Rao Jasti, P. (2015a). Microbial hazards in irrigation water: Standards, norms, and testing to manage use of water in fresh produce primary production. Comprehensive Reviews in Food Science and Food Safety, 14(4), 336–356. https://doi.org/10.1111/1541-4337.12133

Van Haute, S., Sampers, I., Holvoet, K., & Uyttendaelea, M. (2013). Physicochemical quality and chemical safety of chlorine as a reconditioning agent and wash water disinfectant for fresh-cut lettuce washing. Applied and Environmental Microbiology, 79(9), 2850–2861. https://doi.org/10.1128/AEM.03283-12/ASSET/A065B020-E9E1-4378-96A3-19EC0A63D230/ASSETS/GRAPHIC/ZAM9991042880007.JPEG

Vandekinderen, I., Devlieghere, F., De Meulenaer, B., Ragaert, P., & Van Camp, J. (2009). Optimization and evaluation of a decontamination step with peroxyacetic acid for fresh-cut produce. https://doi.org/10.1016/j.fm.2009.06.004

Veschetti, E., Cutilli, D., Bonadonna, L., Briancesco, R., Martini, C., Cecchini, G., Anastasi, P., & Ottaviani, M. (2003). Pilot-plant comparative study of peracetic acid and sodium hypochlorite wastewater disinfection. Water Research, 37(1), 78–94. https://doi.org/10.1016/S0043-1354(02)00248-8

Vital signs: Incidence and trends of infection with pathogens transmitted commonly through food --- Foodborne Diseases Active Surveillance Network, 10 U.S. Sites, 1996--2010. (2011). https://www.cdc.gov/mmwr/preview/mmwrhtml/mm6022a5.htm

Yeni, F., Yavaş, S., Alpas, H., Soyer, Y., Yava¸s, S. Y., Alpas, H., Ye¸, Y., & Soyer, Y. (2016). Most common foodborne pathogens and mycotoxins on fresh produce: A review of recent outbreaks. Http://Dx.Doi.Org/10.1080/10408398.2013.777021, 56(9), 1532–1544. https://doi.org/10.1080/10408398.2013.777021

Zhang, C., Brown, P. J. B., & Hu, Z. (2019). Higher functionality of bacterial plasmid DNA in water after peracetic acid disinfection compared with chlorination. https://doi.org/10.1016/j.scitotenv.2019.05.074

Zhang, K., San, Y., Cao, C., Zhang, T., Cen, C., & Zhou, X. (2020). Optimising the measurement of peracetic acid to assess its degradation during drinking water disinfection. Environmental Science and Pollution Research International, 27(27), 34135–34146. https://doi.org/10.1007/S11356-020-09505-6

Zhang, S., Jiang, L., Li, H., Zhang, J., Sun, T., Dong, Y., & Ding, N. (2022). Disinfection kinetics of peracetic acid inactivation of pathogenic bacteria in water. *Water Cycle*, *3*, 79–85. https://doi.org/10.1016/J.WATCYC.2022.05.002